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(54) Title: PARATHYROID HORMONE RECEPTOR AND DNA ENCODING SAME

(57) Abstract

DNA encoding a parathyroid hormone receptor; production and isolation of recombinant and synthetic parathyroid hormone receptor polypeptides and fragments; antibodies to parathyroid hormone receptors and receptor fragments; methods for screening candidate compounds for antagonistic or agonistic effects on parathyroid hormone receptor action; and diagnostic and therapeutic methods of these compounds are disclosed.



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PARATHYROID HORMONE RECEPTOR AND DNA ENCODING SAME Background of the Invention

Partial funding of the work described herein was 5 provided by the U.S. Government, which has certain rights to the invention.

The invention relates to endocrine receptors.

A crucial step in the expression of hormonal action is the interaction of hormones with receptors on the plasma membrane surface of target cells. The formation of hormone-receptor complexes allows the transduction of extracellular signals into the cell to elicit a variety of biological responses. For example, binding of a hormone such as follicle stimulating hormone

- 15 (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), and chorionic gonadotropin (CG), to its cell surface receptor induces a conformational change in the receptor, resulting in the association of the receptor with a transductor molecule, the stimulatory
- guanine nucleotide (GTP) binding protein, a component of which is (G_s). This association stimulates adenylate cyclase activity which in turn triggers other cellular processes such as protein phosphorylation, steroid synthesis and secretion, and the modulation of ion flux.
- Binding of other hormones, including arginine vasopressin (VP), angiotensin II, and norepinephrine, to their cell surface receptors results in the activation of other types of GTP binding proteins components such as (G_p) , which in turn stimulates the activity of the enzyme
- 30 phospholipase C. The products of phospholipase C hydrolysis initiate a complex cascade of cellular events, including the mobilization of intracellular calcium and protein phosphorylation.

Parathyroid hormone (PTH) is a major regulator of 35 calcium homeostasis whose principal target cells occur in

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bone and kidney. Regulation of calcium concentration is necessary for the normal function of the gastrointestinal, skeletal, neurologic, neuromuscular, and cardiovascular systems. PTH synthesis and release 5 are controlled principally by the serum calcium level: a low level stimulates and a high level suppresses both the hormone synthesis and release. PTH, in turn, maintains the serum calcium level by directly or indirectly promoting calcium entry into the blood at three sites of 10 calcium exchange: gut, bone and kidney. PTH contributes to net gastrointestinal absorption of calcium by favoring the renal synthesis of the active form of vitamin D. promotes calcium resorption from bone by inhibiting osteoblasts and, indirectly, by stimulating 15 differentiation of the bone-resorbing cells, osteoclasts. It also mediates at least three main effects on the kidney: stimulation of tubular calcium reabsorption, enhancement of phosphate clearance, and promotion of an increase in the enzyme that completes synthesis of the 20 active form of vitamin D. PTH exerts these effects primarily through receptor-mediated activation of adenylate cyclase, although receptor-mediated activation of phospholipase C by PTH has also been reported (Hruska et al., J. Clin. Invest. 79:230, 1987).

Disruption of calcium homeostasis may produce many clinical disorders (e.g., severe bone disease, anemia, renal impairment, ulcers, myopathy, and neuropathy) and usually results from conditions which produce an alteration in the level of parathyroid hormone. 30 Hypercalcemia is a condition which is characterized by an elevation in the serum calcium level. It is often associated with primary hyperparathyroidism in which an excess of PTH production occurs as a result of a lesion (e.g., adenoma, hyperplasia or carcinoma) of the 35 parathyroid glands. Another type of hypercalcemia,

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humoral hypercalcemia of malignancy (HHM), is the most common paraneoplastic syndrome. It appears to result in most instances from the production by tumors (e.g., squamous, renal, ovarian or bladder carcinomas) of a 5 novel class of protein hormone which shares amino acid homology with PTH. These PTH-related proteins (PTHrP) appear to mimic certain of the renal and skeletal actions of PTH and are believed to interact with the PTH receptor in these tissues. PTHrP is normally found at low levels in many tissues, including keratinocytes, brain, pituitary, parathyroid, adrenal cortex, medulla, fetal liver, osteoblast-like cells and lactating mammary tissues. In many HHM malignancies, PTHrP is found in the circulatory system at high levels, thereby producing the elevated calcium levels associated with HHM.

Summary of the Invention

The invention features isolated DNA comprising a DNA sequence encoding a cell receptor, preferably a parathyroid hormone receptor, of a vertebrate animal, 20 which receptor has an amino acid sequence with at least 30% (preferably at least 50%, even more preferably at least 60%, and most preferably at least 75%) identity to the amino acid sequence shown in FIG. 3 (SEQ ID NO.: 3): i.e., when the closest match is made between the two 25 amino acid sequences (using standard methods), at least 30% of the amino acid residues of the former sequence are identical to the amino acid residues of the latter sequence. By "isolated" is meant that the DNA is free of the coding sequences of those genes that, in the 30 naturally-occurring genome of the organism (if any) from which the DNA of the invention is derived, immediately flank the gene encoding the DNA of the invention. The isolated DNA may be single-stranded or double-stranded, and may be genomic DNA, cDNA, recombinant hybrid DNA, or

synthetic DNA. It may be identical to a naturallyoccurring, cell receptor- (e.g. PTH receptor) encoding DNA sequence, or may differ from such sequence by the deletion, addition, or substitution of one or more 5 nucleotides. Single-stranded DNAs of the invention are generally at least 8 nucleotides long, (preferably at least 18 nucleotides long, and more preferably at least 30 nucleotides long) ranging up to full length of the gene or cDNA; they preferably are detectably labelled for 10 use as hybridization probes, and may be antisense. Preferably, the isolated DNA hybridizes under conditions of high stringency to all or part of the DNA sequence show in FIG. 1 (SEQ ID NO.:1), FIG. 2 (SEQ ID NO.:2), FIG. 3 (SEQ ID NO.:3), or FIG. 6 (SEQ ID NO.:4). 15 "high stringency" is meant, for example, conditions such as those described herein below for the isolation of human kidney PTH receptor cDNA (also see Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989, hereby incorporated by reference). Most 20 preferably, the animal is a mammal (such as an opossum, a rat, or a human), and the DNA sequence encodes substantially all of the amino acid sequence shown in FIG. 1 (SEQ ID NO.:1), FIG. 2 (SEQ ID NO.:2), FIG. 3 (SEQ ID NO.:3) or FIG. 6 (SEQ ID NO.:4); or is encoded by the 25 coding sequence of one of the plasmids deposited with the American Type Culture Collection (ATCC) and designated ATCC Accession No. 68570 or 68571. The DNA of the invention may be incorporated into a vector [which may be provided as a purified preparation (e.g., a vector 30 separated from the mixture of vectors which make up a library)] containing a DNA sequence encoding a cell receptor of the invention (e.g. parathyroid hormone receptor) or fragment of the receptor, and a cell or essentially homogenous population of cells (e.g., 35 prokaryotic cells, or eukaryotic cells such as mammalian

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cells) which contain the vector (or the isolated DNA described above). By "essentially homogenous" is meant that at least 99% of the cells contain the vector of the invention (or the isolated DNA, as the case may be).

5 Preferably, this vector (e.g., R15B) is capable of directing expression of a parathyroid hormone receptor (for example, in a cell transfected or transformed with the vector).

In another aspect, the invention features a cell
receptor, preferably parathyroid hormone receptor, (or an
essentially purified preparation thereof) produced by
expression of a recombinant DNA molecule encoding the
cell receptor. An "essentially purified preparation" is
one which is substantially free of the proteins and
lipids with which it is naturally associated.

In a related aspect, the invention features a polypeptide which includes a fragment of a naturally-occurring cell receptor of the invention. Preferably, the polypeptide includes a fragment of a naturally-occurring parathyroid hormone receptor which is capable of binding parathyroid hormone or parathyroid hormone-related protein. In preferred embodiments, this fragment is at least six amino acids long, and has a sequence selected from the group including:

- 25 (a) TNETREREVFDRLGMIYTVG; (SEQ ID NO.: 5)
 - (b) YLYSGFTLDEAERLTEEEL; (SEQ ID NO.: 6)
 - (c) VTFFLYFLATNYYWILVEG; (SEQ ID NO.: 7)
 - (d) Y-RATLANTGCWDLSSGHKKWIIQVP; (SEQ. ID NO.: 8)
 - (e) PYTEYSGTLWQIQMHYEM; (SEQ ID NO.: 9)
- 30 (f) DDVFTKEEQIFLLHRAQA; (SEQ ID NO.: 10)
 - (g) FFRLHCTRNY; (SEQ ID NO.: 11)
 - (h) EKKYLWGFTL; (SEQ ID NO.: 12)
 - (i) VLATKLRETNAGRCDTRQQYRKLLK; or (SEQ ID NO. 13)
- (j) a fragment (i.e., a portion at least six
 35 residues long, but less than all) or analog of (a) (i)

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which is capable of binding parathyroid hormone or parathyroid hormone-related protein [wherein "analog" denotes a peptide having a sequence at least 50% (and preferably at least 70%) identical to the peptide of which it is an analog]. Preferably, the polypeptide of the invention is produced by expression of a recombinant DNA molecule or is synthetic (i.e., assembled by chemical rather than biological means). The invention provides a method for producing such a polypeptide, which method includes providing a cell containing isolated DNA encoding a cell receptor of the invention or receptor fragment and culturing this cell under conditions which permit expression of a polypeptide from the isolated DNA.

The invention also features an antibody 15 (monoclonal or poylclonal), and a purified preparation of an antibody, which is capable of forming an immune complex with a cell receptor of the invention (preferably a parathyroid hormone receptor such as a human PTH receptor) such antibody being generated by using as 20 antigen either (1) a polypeptide that includes a fragment of the cell receptor of the invention, or (2) a cell receptor of the invention which is on the surface of a cell. This antibody is preferably capable of neutralizing (i.e., partially or completely inhibiting) a 25 biological activity of the cell receptor of the invention (i.e., a component of one of the cascades naturally triggered by the receptor when its ligand binds to it). In preferred embodiments, the antibody of the invention is capable of forming an immune complex with parathyroid 30 hormone receptor and is capable of neutralizing a biological activity of the PTH receptor (i.e. adenylate cyclase activation or phospholipase C stimulation)

Also within the invention is a therapeutic composition including, in a pharmaceutically-acceptable carrier, (a) a cell receptor of the invention, (b) a

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polypeptide containing a fragment of the cell receptor of the invention, or (c) an antibody to a cell receptor of the invention. These therapeutic compositions provide a means for treating various disorders characterized by overstimulation of the cell receptors of the invention by their ligand. In preferred embodiments, the polypeptides of the invention include the PTH receptor, fragments of the PTH receptor and antibodies which form immune complexes with the PTH receptor. These polypeptides and antibodies are useful as diagnostics, for distinguishing those cases of hypercalcemia related to PTH or PTHrP from those which are not.

The nucleic acid probes of the invention enable one of ordinary skill in the art of genetic engineering to identify and clone cell receptor homologs or cell receptors from any species which are related to the cell receptors of the invention, expanding the usefulness of the sequences of the invention.

Other features and advantages of the invention 20 will be apparent from the following description of the preferred embodiments and from the claims.

<u>Detailed Description</u>

The drawings will first be briefly described. <u>DRAWINGS</u>

FIG. 1 is a representation of the nucleic acid and amino acid sequence encoding the opossum kidney PTH/PTHrP receptor clone, OK-H. (SEQ ID NO.: 1)

FIG. 2 is a representation of the nucleic acid and amino acid sequence encoding the opossum kidney PTH/PTHrP receptor clone, OK-O. (SEQ ID NO.: 2)

FIG. 3 is a representation of the nucleic acid and amino acid sequence encoding the rat bone PTH/PTHrP receptor clone, R15B. (SEQ ID NO.: 3)

FIG. 4 is a comparison of the deduced amino acid sequences encoded by cDNAs from clones OK-O and R15B.

FIG. 5 is a comparison of the deduced amino acid sequences of OK-O, OK-H and R15B, lined up according to 5 sequence homology.

FIG. 6 is a representation of the nucleic acid and amino acid sequence encoding the human PTH/PTHrP receptor. (SEQ ID NO.: 4)

FIG. 7 is a schematic representation of the rat bone PTH/PTHrP receptor cDNA, the human genomic DNA clone HPG1 and two cDNA clones encoding the human PTH/PTHrP receptor.

FIG. 8 is a hydrophobicity plot of the deduced amino acid sequence of the human kidney PTH/PTHrP receptor. Predicted membrane-spanning domains I through VII are indicated; A, B and C indicate additional hydrophobic regions.

FIG. 9 is a graph illustrating binding of PTHrP to COS cells transfected with OK-H.

FIG. 10 is a graph illustrating stimulation of intracellular free calcium by NlePTH in COS cells transfected with OK-H.

FIG. 11 is a graph illustrating binding of PTHrP to COS cells transfected with OK-O.

FIG. 12 is a graph illustrating stimulation of intracellular free calcium by NlePTH in COS cells transfected with OK-O.

FIG. 13 is a graph illustrating binding of PTHrP to COS cells transfected with R15B.

FIG. 14 is a graph illustrating stimulation of intracellular free calcium by NlePTH in COS cells transfected with R15B.

FIG. 15 is a graph illustrating stimulation of inositol phosphate metabolism by NlePTH in COS cells transfected with OK-H, OK-O, or R15B.

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FIG. 16 is a graph illustrating cyclic AMP accumulation in COS cells transfected with CDM-8, OK-H, R15B by NlePTH.

FIG. 17 are graphs illustrating binding of 125_I-5 labelled PTH(1-34) (A and B) and 125_I-labelled PTHrP(1-36) (C and D) to COS-7 cells transiently expressing the human kidney (A and C) and the rat bone (B and D) PTH/PTHrP receptor; competing ligands included PTH(1-34) (D), PTHrP(1-36) (*), PTH(3-34) (D), PTH(7-34) (+).

10 Data are given as % specific binding and represent the mean±SD of at least three independent experiments.

FIG. 18 is a bar graph illustrating stimulated accumulation of intracellular cAMP in COS-7 cells transiently expressing the human kidney receptor. Data show the mean±SD, and are representative of at least three independent experiments.

FIG. 19 represents a Northern blot analysis of total RNA (~ 10 μg/lane) prepared from human kidney (A) and SaOS-2 cells (B). The blot was hybridized with the full length cDNA encoding the human kidney PTH/PTHrP receptor; positions of 28S and 18S ribosomal RNA bands are indicated.

FIG. 20 represents a Southern blot analysis of human genomic DNA digested with SstI, HindIII, and XhoI
 25 (~ 10μg/lane. The blot was hybridized with the full length cDNA encoding the human kidney PTH/PTHrP receptor.

FIG. 21 is a schematic diagram of the proposed arrangement, in a cellular membrane, of PTH/PTHrP rat bone receptor encoded by R15B.

MATERIALS AND METHODS

GENERAL: [Nle^{8,18}, Tyr³⁴]bPTH(1-34)amide (PTH(1-34)),

[Nle^{8,18}, Tyr³⁴]bPTH(3-34)amide (PTH(3-34)), and [Nle^{8,18},

Tyr³⁴]bPTH(7-34)amide (PTH(7-34)) were obtained from

Bachem Fine Chemicals, Torrance, CA; [Tyr³⁶]PTHrP(1-

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36) amide (PTHrP(1-36)) was synthesized as described (Keutman et al., Endocrinology 117:1230, 1985) using an Applied Biosystems Synthesizer 420A. Dulbecco's modified Eagles medium (DMEM), EDTA/trypsin, and gentamycin were from GIBCO (Grand Island, NY); fetal bovine serum (FBS) was from Hiclone Laboratory, Logan, UT. Total RNA from human kidney was provided by Per Hellman, University Hospital, Uppsala, Sweden. Oligonucleotide primers were

synthesized using an Applied Biosystems 380B DNA

Synthesizer. Restriction enzymes, Klenow enzyme, T4

polynucleotide Kinase and T4 DNA ligase were from New
England Biolabs, Beverly, MA. Calf alkaline phosphatase
was from Boehringer Mannheim, Germany. All other reagents
were of highest purity available.

15 CELLS

Cell lines used include COS cells, OK cells, SaOS
2 cells, CHO cells, AtT20 cells, LLC-PK1 cells, and UMR
106 cells, which are available from a variety of sources including the American Type Culture Collection (Rockland,

20 Maryland), Accession Nos. CRL1650, CRL6551, HTB85, CCL61, CCL89, CL101, and CRL1161, respectively. ROS 17/2 and ROS 17/2.8 are available from a number of sources including Dr. Gideon Rodan (Merck Laboratories, West Point, PA). MC-3T3 cells are derived from mouse bone

25 cells and are also available from a number of sources including Dr. Chohei Shigeno (Dept. of Biochem. Medicine, Hyoto Univ., Kyoto, Japan).

All cells were grown in a humidified 95% air, 5% CO₂ atmosphere and maintained in monolayer culture with 30 Ham's

F-12 or DMEM medium (Grand Island Biological Co.), supplemented with 5% or 10% fetal calf serum (M.A. Bioproducts, Walkersville, MD). The medium was changed every 3 or 4 days, and the cells were subcultured every 2 or 3 weeks by

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trypsinization using standard methods. CLONING

Isolation of cDNA clones encoding the rat and opossum PTH/PTHrP receptors: Total RNA was initially 5 isolated from rat osteosarcoma (ROS) cells (ROS 17/2.8) and opossum kidney (OK) cells, by standard methods using guanidium isothiocyanate (Ullrich et al., Science 196: 1313, 1977; Chirgwin et al. Biochemistry 24: 5294, 1979), and centrifugation through cesium chloride (Gilsen et 10 al., Biochemistry 13: 2633, 1974). Poly A+ RNAs (mRNAs) were then recovered after passage of the total RNAs over oligo dT columns (Pharmacia, Piscataway, NJ) by the method of Aviv and Leder (Proc. Natl. Acad Sci. USA 69: 14087, 1972). The cDNA library from the ROS 17/2.8 mRNA 15 was prepared from poly A+ RNA using the method of Gubler and Hoffman (Gene (Amst.) $\underline{25}$: 263, 1983). Oligo dTprimed and random-primed cDNAs were synthesized from poly A+ ROS 17/2.8 and OK cell mRNA, respectively (Aviv and Leder, supra). The cDNAs were ligated to BstX1 linkers 20 (Invitrogen, San Diego, CA) and size-selected by centrifugation (3 h, 55,000 xg) in a 5-20% potassium acetate gradient. The size-selected cDNA was then inserted into the plasmid vector, pcDNA I (Invitrogen), using the non-self annealing BstX1 restriction sites. 25 The resultant plasmid libraries were then used to transform E. coli (MC1061/P3, Invitrogen) containing a larger helper plasmid, p3. The p3 plasmid possesses amber mutations in two genes which code for ampicillin and 30 tetracycline resistance. Using ampicillin and tetracycline selection, only those cells containing both the p3 and a tRNA suppressor gene, which is contained within pcDNA I, were capable of growth. The transformed bacteria were then grown to confluence, and the plasmid

35 DNAs isolated using standard techniques (e.g., see

Ausebel et al., Current Protocols in Molecular Biology, John Wiley Sons, New York, 1989). These DNAs were then taken up in a DEAE-dextran solution, and used to transfect African Green Monkey kidney (COS) cells, which had been grown to 75% confluence in "sideflasks" (Nunc, Denmark).

Screening for COS cells containing plasmids capable of expressing functionally-intact ROS or OK cell parathyroid hormone/parathyroid hormone related-protein 10 (PTH/PTHrP) receptor proteins was performed according to Gearing et al. (EMBO J. 8: 3676, 1989), with some minor modifications including DEAE-Dextran transfection in sideflasks. Forty-eight hours after transfection, the cells were tested for binding of 125I-labeled [Tyr36]PTHrp 15 (1-36) amide, using methods previously described (Yamamoto et al., Endocrinology 122: 1208, 1988), with the following exceptions: the time and temperature of the incubation were 2h and room temperature, respectively. After rinsing, the cells were fixed with 1.25% 20 glutaraldehyde, and rinsed with 1% gelatin. After snapping off the top of the sideflask, the remaining microscope slide was dipped into NTB-2 photographic emulsion (Eastman Kodak, Rochester, NY). After 3-4 days of exposure at 4°C, the slides were developed, fixed, and 25 stained with 0.03% toluene blue. Screening of each slide was performed under a light microscope (Olympus). One pool of plasmid-DNA from ROS cells, and two pools of plasmid-DNA from OK cells, (10,000 independent clones), each gave rise to 3-4 transfected COS cells expressing 30 the PTH/PTHrP receptor. These pools were subsequently subdivided. The subpools were used to transfect COS cells, and single clones were identified that expressed receptor protein capable of binding the radioligand.

Isolation of cDNA and genomic DNA clones encoding
the human PTH/PTHrP receptor: A human kidney oligo dT-

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primed cDNA library (1.7x106 independent clones) in lambda GT10 and a genomic library of human placental DNA (2.5x10⁶ independent clones) in EMBL3 (Sp6/T7) (Clontech, Palo Alto, CA) were screened by the plaque hybridization 5 technique (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed. pp. 108-113, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989) with the 32P-labelled (random primed labelling kit Boehringer Mannheim, Germany) BamHI/NotI 1.8kb restriction enzyme 10 fragment encoding most of the coding sequence of the rat bone PTH/PTHrp receptor (Fig. 3). The nitrocellulose filters were incubated at 42°C for 4 hrs in a prehybridization solution containing 50% formamide, 4x saline sodium citrate (SSC; 1x SSC: 300 mM NaCl, 30 mM 15 NaCitrate, pH 7.0), 2x Denhardt's solution, 10% Dextran sulphate, 100 μ g/ml salmon sperm DNA (final concentration). The hybridizations were carried out in the same solution at 42°C for 18-24h. Filters were washed with 2x SSC/0.1% SDS for 30 minutes at room 20 temperature and then with 1x SSC/0.1% SDS for 30 minutes at 45°C. The films were exposed at -80°C for 18-24h using intensifying screens.

About 1,000,000 clones were screened from each library. Positive clones were plaque-purified and lambda phage DNA was isolated (Sambrook et al., supra). Cloned inserts were removed from phage DNA by digestion with restriction endonucleases HindIII and EcoRI (lambda GT10 library), or with XhoI and SstI (EMBL3 library), and were then subcloned into pcDNAI (Invitrogen, San Diego, CA) using the appropriate, dephosphorylated restriction sites. Sequencing of the CsCl2-purified subclones was performed according to Sanger et al. (Biochem 74:5463, 1977) by the dideoxy termination method (Sequenase version 2 sequencing kit, United States Biochemical Corporation, Cleveland, OH).

Reverse transcription and polymerase chain reaction (PCR): 3 μg of poly (A)+ RNA from human kidney (Clontech, Palo Alto, CA) in 73.5 μl of H₂O was incubated at 100°C for 30 seconds, quenched on ice, and then added to 20 μl of 5x RT buffer (1x RT buffer: 40 mM Tris-HCl, pH 8.2, 40 mM KCl, 6.6 mM MgCl₂, 10 mM dithiothreitol, and dNTPs at 0.5 mM each), 2 μl (4 units) RNasin (Promega Biotec, Madison, WI), 1 μl (80 pmo/μl) of the human cDNA primer H12

10 (5'-AGATGAGGCTGTGCAGGT-3'; SEQ ID NO.: 14) and 80 units of avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL). The reaction mixture was incubated for 40 minutes at 42°C. One-tenth of the first strand synthesis reaction mixture was then amplified by PCR in a final volume of 100 μl containing 3 mM MgSO₄, 200 μM dNTPs, 2 units of Vent polymerase (New England Biolab, Beverly, MA), and 2 μM each of the forward and the reverse primers (PCR conditions: denaturing for 1 min at 94°C, annealing for 1 min at 50°C, and extension at 72°C for 3 minutes; 40 cycles).

Two independent PCRs were performed using two different forward primers: i) degenerate primer RK-1 (5'-GGAATTCCATGGGAGCGGCCCGGAT-3'; SEQ ID NO.: 15) based

the 5' coding end of the two previously cloned PTH/PTHrP receptors (described above), and ii) primer RK-2 (5'-CGGGATCCCGCGGCCCTAGGCGGT-3'; SEQ ID NO.: 16) based on the 5' untranslated region of the human genomic clone HPG1. Both PCR reactions used the reverse primer H26 (5'AGTATAGCGTCCTTGACGA-3'; SEQ ID NO.: 17) representing nucleotides 713 to 731 of the coding region of the human PTH/PTHrP receptor (Fig. 4). PCR products were bluntended using Klenow enzyme and cloned into dephosphorylated pcDNAI cut with EcoRV.

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Northern blot analysis: Total RNA was extracted from SaOS-2 cells and from human kidney by the guanidine thiocyanate method (Chirgwin et al., Biochem. 18:5294, 1979). For Northern blot analysis, ~10 μg of total RNA 5 was subjected to electrophoresis on a 1.5%/37% formaldehyde gel and blotted onto nitrocellulose filters (Schleicher and Schuell, Keene, NH). The hybridization conditions were the same as those for screening the phage libraries (see above). The filters were washed at a final 10 stringency of 0.5x SSC/0.1% SDS for 30 min at 60°C and exposed for autoradiography.

Southern blot analysis: Human genomic DNA was prepared using the SDS/proteinase K method (Gross-Bellard et al., Eur. J. Biochem. 36:32, 1973). For Southern 15 analysis, ~10 μ g of DNA was digested with SstI, PvuII and XhoI; subjected to electrophoresis on a 0.8% agarose gel; and blotted onto nitrocellulose membranes (Schleicher and Schuell, Keene, NH). The hybridization conditions were the same as those for screening the phage libraries (see 20 above). The filters were washed at a final stringency of 0.5x SSC/0.1% SDS for 30 min at 55°C and exposed for autoradiography.

FUNCTIONAL ASSAYS

Tests to characterize the functional properties of 25 the cloned receptors expressed on COS cells included:

I) binding of PTH and PTHrP fragments and II) stimulation of cyclic AMP accumulation by analoques, PTH and PTHrP fragments and analogues,

III) increase of intracellular free calcium by PTH 30 and PTHrP fragments and analogues, and

IV) activation of inositol phosphate metabolism by PTH and PTHrP fragments and analogues. The methodologies are as follows:

Radioreceptor Assay

[Nle8,Nle18,Tyr34]bPTH-(1-34)amide (NlePTH), and [Tyr36]PTHrP(1-36)amide(PTHrP) were iodinated with Na125I (carrier free, New England Nuclear, Boston, MA) as 5 previously reported (Segre et al., J. Biol. Chem. 254: 6980, 1979), and purified by reverse-phase HPLC. brief, the labeled peptide was dissolved in 0.1% trifluoracetic acid (TFA), applied to a C18 Sep-pak cartridge (Waters Associates, Inc., Milford, MA) and 10 eluted with a solution of 60% acetonitrile in 0.1% TFA. After lyophilization, the radioligand then was applied to C₁₈-μBondapak column (3.9 mm x 30 cm. Waters Associates) and eluted over 30 min with a linear gradient of 30-50% acetonitrile-0.1% TFA at a flow rate of 2 ml/min. The 15 radioligand eluted in two peaks; the first peak, which eluted at approximately 38% acetonitrile, was used in these studies because it gave higher total and specific .bindings. The specific activity was 500 ± 75 mCi/mg, which corresponds to an average iodine-peptide ratio of

COS-7 cells were grown in 15 cm plates in DMEM, 10% heat-inactivated FBS, 10 mg/L gentamycin until 80-90% confluent. Twenty-four hours after transfection by the

- DEAE/Dextran method (Sambrook et al., supra), with 1-2 μ g of plasmid DNA, the cells were trypsinized and replated in multiwell plastic dishes (16 or 35 mm diameter, Costar, Cambridge, MA) at a cell concentration of 5 x 10^4 cells/cm²). Cell number increased only slightly after
- 30 transfection. After continuing culture for another 48 h, radiorecepter assays were performed. The culture medium was replaced with buffer containing 50 mM Tris-HCL (pH 7.7),

100 mM NaCl, 2 mM CaCl₂, 5 mM KCL, 0.5% heat-inactivated 35 fetal bovine serum (GIBCO), and 5% heat-inactivated horse

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serum (KC Biological Inc., Lenexa, KS) immediately before studies were initiated. Unless otherwise indicated, studies were conducted with cells incubated in this buffer at 15°C for 4 h with 4 x 10⁵ cpm/ml (9.6 x 10⁻¹¹ M) of ¹²⁵I-labeled NlePTH or PTHrP.

Incubations were terminated by aspirating the buffer, and repeatedly (x3) washing the culture dishes containing the adherent cells with chilled 0.9% NaCl solution, over a 15 sec period. Cell-bound radioactivity 10 was recovered by the sequential addition (x3) of 1 N NaOH (200 μl) to each well. After 30 min at room temperature, the NaOH was transferred to a glass tube. A second and third extraction with 1 N NaOH (200 μl) were combined with the first, and the total radioactivity was 15 counted in a γ-spectrometer (Packard Instruments, Downers Grove, IL). Tracer adherence to culture vessel without cells was negligible (<0.2% of total counts added), if vessels were preincubated with culture medium. Determinations of CAMP accumulation

Intracellular cAMP accumulation was measured as 20 described previously (Abou-Samra et al., J. Biol. Chem. 262:1129, 1986). Cells in 24-well plates were rinsed with culture medium containing 0.1% BSA and 2mM IBMX. The cells were then incubated with PTH or PTHrP for 15 25 min. at 37° C. The supernatant was removed and the cells immediately frozen by placing the whole plate in dry ice powder. Intracellular cAMP was extracted by thawing the cells in 1ml of 50 mM HCl and analyzed by a specific radioimmunoassay using an anti-cAMP antibody (e.g., 30 Sigma, St. Louis, MO). A cAMP analog (2'-0-monosuccinyladenosine 3':5'-cyclic monophosphate tyrosyl methyl ester, obtained from Sigma) which was used a tracer for cAMP was iodinated by the chloramine T method. Free iodine was removed by adsorbing the iodinated cAMP analog

35 onto a C18 Sep-pak cartridge (Waters, Milford, MA).

After washing with dH20, the iodinated cAMP analog was eluted from the Sep-pak Cartridge with 40% acetonitrille (ACN) and 0.1% trifluoroacetic acid (TFA). The iodinated cAMP analog was lyophilized, reconstituted in 1 ml 0.1% 5 TFA, and injected into a C18 reverse phase HPLC column (Waters). The column was equilibrated with 10% ACN in 0.1% TFA, and eluted with gradient of 10-30% ACN in 0.1% This allows separation of the mono-iodinated cAMP analog from the non-iodinated cAMP analog. The tracer is 10 stable for up to 4 months when stored at -20° C. standard used for the assay, adenosine 3':5'-cyclic monophosphate, was purchased from Sigma. Samples (1-10 μ l of HCl extracts) or standards (0.04-100 fmol/tube) were diluted in 50 mM Na-acetate (pH 5.5), and acetylated 15 with 10 μ l of mixture of triethylamine and acetic anhydride (2:1 vol:vol). After acetylation, cAMP antiserum (100 μ l) was added from a stock solution (1:4000) made in PBS (pH 7.4), 5 mM EDTA and 1% normal rabbit serum. The tracer was diluted in PBS (pH 7.4) 20 with 0.1% BSA, and added (20,000 cpm/tube). The assay was incubated at 4° C overnight. The bound tracer was precipitated by adding 100 μ l of goat anti-rabbit antiserum (1:20 in PBS) and 1 ml of 7% polyethyleneglycol (MW 5000-6000), centrifuging at 2000 rpm for 30 min. at 25 4° C. The supernatant was removed and the bound radioactivity was counted in a η -counter (Micromedic). Standard curves were calculated using the four-parameter RIA program supplied by Micromedic. Typically, the assay sensitivity is 0.1 fmol/ tube, and the standard 30 concentration that displaces 50% of tracer is 5 fmol/tube.

In an alternative method for assaying cAMP accumulation, COS cells transfected with PTH/PTHrP receptor cDNA are harvested with a plastic policeman into a solution containing 10 mM Tris-HCl (pH 7.5), 0.2 mM

- 19 -MgCl, 0.5 mM ethyleneglycolbis(β -amino ethyl ether) N,N'tetra-acetic acid (EGTA) (Sigma) and 1 mM dithiothreitol (Sigma). Cells are homogenated by 20 strokes of tightlyfitting Dounce homogenizer, and centrifuged at 13,000 x g5 for 15 min at 4°C (Eppendorf, type 5412, Brinkmann Instruments, Inc., Westburg, NY). The pellet containing the plasma membranes is resuspended in the same buffer by several strokes with a Dounce homogenizer, and further diluted with the same buffer to a protein concentration 10 of approximately 1.2 mg/ml, as determined by the method of Lowry et al., J. Biol. Chem 193: 265, 1951). Approximately 30 μ g (25 μ l) membrane are incubated with varying concentrations of hormone or vehicle alone for 10 min at 37°C (final volume, 100 μ l) 15 in 50 mM Tris-HCl (pH 7.5), 0.8 mM ATP, 4 x 10^6 cpm [α - 32 P] ATP (New England Nuclear, Boston, MA), 9 mM theophylline, 4.2 mM MgCl₂, 26 mM KCl, 0.12% BSA, and an ATP-regenerating system containing 5 mM creatine phosphate (Schwartz/Mann Division, Becton-Dickenson & 20 Co., Orangeburg, NY) and 0.1 mg/ml creatine phosphokinase

- (Shwartz/Mann). Incubations are initiated by addition of the membrane suspension and terminated by addition of 100 μ l of a solution containing 20 mM cAMP, approximately 50,000 cpm [3H]cAMP, and 80 mM ATP. The reaction mixture
- 25 is boiled, and the $[^{32}P]cAMP$ generated is purified by sequential chromatography on ion-exchange columns (Dowex 50 W-X4, Biorad Lab, Richmond, CA) and alumina (Sigma). The $[^{32}P]$ cAMP may be counted in a β -scintillation counter (Packard Instrument Co.), with correction for recovery of 30 [3H]CAMP.

Determination of intracellular free calcium

Measurements of intracellular calcium levels in cells transfected with PTH/PTHrP receptor cDNAs were performed using Fura-2 AM (acetomethoxy ester of Fura-2,

Molecular Probes Inc., Eugene, OR) loaded cells. Details of the methodology are:

Coverslips plated with COS cells were incubated in Fura-2 AM loading buffer containing, in mM: HEPES (N
[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]),
20; CaCl₂, 1; KCl 5; NaCl, 145; MgSO₄, 0.5; NaHCO₃, 25;
K₂HPO₄, 1.4; glucose, 10; and Fura-2 AM 91-(2-5'carboxyoxazol-2'-yl)-6-aminobenzofuran-5oxy-(2'-amino-5'-methylphenoxy)ethane-N,N,N',N'-tetraaecetic acid

- 10 acetomethoxy ester), 0.5; at 37°C at pH7.4, aerated with 95% air and 5% CO₂ for 45 minutes. Cells loaded with Fura-2 AM were then washed with a modified Krebs-Heinseleit (KH) buffer containing, in mM: HEPES, 20; CaCl₂, 1; KCl, 5; NaCl, 145; MgSO₄, 0.5; Na₂HPO₄, 1;
- 15 glucose, 5; pH7.4. To check that cleavage of the ester occurred, the excitation spectra after different times of Fura-2 AM incubation were measured. At 5 min. after the start of incubation, the excitation spectrum peaked at approximately 360 nm, reflecting incomplete hydrolysis of
- 20 Fura-2 AM, whereas beyond 30 min. the excitation spectrum peaked at 345 nM, characteristic of Fura-2.

To measure fluorescence of individual cells, the cover slips were placed in a microscope tissue chamber (Biophysica Technologies, Inc., MD). The chamber consisted of a shallow, sloped compartment made of Teflon with a silicone rubber seal. The cover slips served as

- with a silicone rubber seal. The cover slips served as the bottom of the chamber. A heater/cooler ring was encased in the silicone rubber which sealed the coverslip in place. Temperatures were varied between 22°C and 37°C by applying 0.7 4 % to 10.
- 30 by applying 0-7.4 V to the heater. If the temperature is not specifically stated, the experiment was performed at 37°C. The chamber was mounted on the stage of an inverted microscope (Zeiss IM-35, Thornwood, NY). Fura-2 fluorescence was excited with a 75 watt Xenon arc lamp
- 35 placed at the focal point of a condenser (Photon

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Technologies International (PTI) Inc., NJ). Grating monochromators, alternated by a rotating chopper in which mirror vanes alternate with transmitting sectors, were used for selecting wavelengths. The monochromator outputs were combined to form a common optical path which exited the source housing through an adjustable iris. The light then passed through quartz lenses and a dichroic mirror through a 100x Nikon Fluor objective. A photon-counting PMT device detection was used to measure the light output. Data analysis was performed using PTI software run on an IBM-compatible AT/286 computer using the MS-DOS operating system. Data was retained and manipulated in a packed binary format.

Intracellular calcium concentrations were 15 calculated according to the formula: [Ca²⁺]i=Kd(R-Rmin)/(Rmax-R)B, where R is the ratio of fluorescence of the cell at 340 and 380 nm; Rmax and Rmin represent the ratios of Fura-2 fluorescence intensity at 340 and 380 nm excitation wavelengths in the presence of a saturating 20 amount of calcium and effectively zero calcium, respectively; B is the ratio of fluorescence of Fura-2 at 380 nm in zero calcium to that in saturating amounts of calcium; and K_d is the dissociation constant of Fura-2 for calcium. To determine Rmax, at the end of an 25 experiment ionomycin was added to the Fura-2 AM loaded cells to equilibrate Ca^{2+} between the extracellular (1mM) and intracellular environments. To calculate Rmin, 1mM EGTA was then added to the bathing solution. Different dissociation constants were used at the different 30 temperatures: 224 nM at 34-37°C and 135 nM at 24-27°C.

<u>Determination</u> of inositol phosphate

The level of inositol phosphate metabolism was determined in COS cells transfected with PTH/PTHrP

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receptors using previously published methods (Bonventre, et al., J. Biol. Chem. 265: 4934, 1990).

RESULTS

Molecular characterization

Two independent clones (OK-H and OK-O), both of which were isolated from the OK cell cDNA library, had lengths of approximately 2 kilobases. The determined nucleotide sequence and predicted amino acid sequence of these clones are shown in Figs. 1 (SEQ ID NO.:1) and 2 (SEQ ID NO.:2) respectively. The R15B clone isolated from the ROS cell cDNA library had a length of approximately 4 kilobases. The determined nucleotide sequence and predicted amino acid sequence of the rat bone PTH/PTHrP receptor is depicted in Fig. 3 (SEQ ID NO.:3).

The three cDNA clones appear to be full-length by the criteria of having codons encoding methionine residues that are predicted to be the likely candidates as initiator methionines. These methionine codons are followed by amino acid sequences (deduced from the DNA) with properties suggesting that they are "signal-peptide" sequences. All three receptor cDNAs have stop codons at locations that permit these receptors to "fit" a putative seven-membrane spanning model, a model typical for G-protein-linked receptors. Most importantly, all three cloned receptors bind ligands and, when activated, are capable of activating intracellular effectors. These properties suggest that all three of the isolated clones encode full-length cDNAs.

Fig. 4 demonstrates the high degree of homology between the amino acid sequences encoded by the cDNAs from OK-O and ROS 15B. There is an overall 87% homology and a 77.8% amino acid identity between these two receptors. This high level of identity over long

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stretches of amnio acids d monstrates that the amino acid sequence of the PTH receptor is evolutionarily conserved to a high degree. This allows the data from both OK-O and R15B to be extrapolated to other species, including 5 human.

Fig. 5 shows the deduced amino acid sequences of all three cloned cDNAs lined up according to sequence homology. The OK-H sequence is identical to OK-O except in the C-terminus tail, where the OK-O sequence totals 10 585 amino acids whereas the OK-H sequence stops at 515 amino acids. This difference is attributable to a single nucleotide (G) deleted in the OK-H sequence compared to the OK-O sequence, causing a frame shift and early stop codon in the former. It is not known whether OK-O and 15 OK-H represent the products of two separate genes or of a laboratory artifact.

Some G-protein-coupled receptors are encoded by intronless genes (Kobilka et al., Nature 329:75, 1987); Kobilka et al., J. Biol. Chem. 262:7321, 1987; Heckert et 20 al., Mol. Endocrinol. 6:70, 1992; Kobilka et al., Science 238:650, 1987; Bonner et al., Science 237:527, 1987; Sunahara et al., Nature 347:80, 1990). To isolate a human PTH/PTHrP receptor cDNA, both a human cDNA library and a human genomic library were screened with a probe 25 (BamHI/NotI) representing most of the coding region of the rat bone PTH/PTHrP receptor (Fig. 3). Screening the human kidney cDNA library led to the isolation of the clone HK-1 (Fig. 6) [SEQ ID NO.: 6]. Since one of the two EcoRI cloning sites of lambda GT10 proved to be 30 eliminated as a result of the library construction, the HindIII/EcoRI phage fragment containing the cDNA insert and ~250 bp of the 37 kb (left) lambda arm was subcloned into the corresponding restriction sites in pcDNAI. DNA sequencing revealed that the cloned cDNA contained ~1000 35 bp of the 3' coding region and ~200 bp of the 3' noncoding region including an A-rich 3' end. The coding region 5' to the XhoI site was subsequently used to rescreen the library and led to the isolation of the clone HK-2 which, after subcloning into pcDNAI, proved to contain ~1400 bp of the coding region. For the third screening of the library, the PvuII/PstI fragment of HK-2 was used; the isolated clone HK-3 proved to be identical to HK-2.

The genomic library screening (~10⁶ pfu) resulted in the isolation of four independent clones. Comparison of Southern blot analyses of restriction enzyme digests of these clones with that of normal genomic DNA, revealed that one 15 kb genomic clone, HPG1 (also referred to as HG4A), contained a SstI/SstI fragment that had the same

- 15 size as one hybridizing DNA species from normal human genomic DNA digested with SstI (see below). The hybridizing 2.3 kb SstI/SstI DNA fragment and an ~8 kb XhoI fragment which comprised the SstI/SstI fragment were both subcloned into pcDNAI. Further Southern blot
- analysis of the SstI/SstI DNA fragment revealed that an ~1000 bp BamHI/SstI fragment encoded a portion of the human PTH/PTHrP receptor which later proved to represent the exon encoding the putative signal peptide and the 5' non-translated region which is interrupted by an ~1000 bp intron (Fig. 7).

To isolate the remaining ~450 nucleotides of the coding region, poly (A)+ RNA from human kidney was reverse transcribed after priming with H12 (Fig. 7). After single strand synthesis, two independent PCRs were performed using two different forward primers: i) a degenerate primer RK- 1 based on the 5' coding end of the two previously cloned PTH/PTHrP receptors, OK-O and R15B; and ii) primer RK-2 based on the 5' non-coding region of HPG1. H-26 was used as the reverse primer for both reactions. Southern blot and restriction map analyses

confirmed the expected size of the amplified DNA encoding the human PTH/PTHrP receptor. The blunt-ended PCR products encoding the 5' end of the human PTH/PTHrP were cloned into pcDNAI using the dephosphorylated EcoRV 5 sites. Sequence analysis of each PCR clone confirmed their 5' nucleotide difference due to the difference in forward primer sequence, but revealed otherwise identical sequences. Nucleotide sequencing of both strands of the human PTH/PTHrP receptor cDNA revealed an open reading 10 frame encoding a 593-amino acid protein (Fig. 6, SEQ ID NO.:4).

The full-length human kidney PTH/PTHrP receptor cDNA, HKrk, was constructed using the BamHI/PvuII fragment of PCR clone #2 and HK-2. Using the full-15 length cDNA encoding the human PTH/PTHrP receptor, Northern blot analysis of total RNA (~10 µg/lane) from human kidney and SaOS-2 cells revealed one major hybridizing DNA species of ~2.5 kb (Fig. 19). The XhoI digest of normal human genomic DNA, when probed with the 20 same full-length cDNA (Fig. 20), revealed one major hybridizing species of about 5.5 kb, and two DNA species of 4 and 8 kb which weakly hybridized. These date suggest that the human PTH/PTHrP receptor is the product of a single gene. This full-length clone was then 25 transiently expressed in COS-7 cells for functional and biological characterization by the methods cited above.

Comparison of the human receptor with the opposum kidney PTH/PTHrP receptor and the rate bone PTH/PTHrP receptor, revealed 81% and 91% amino acid sequence 30 identity, respectively, and consequently a very similar hydrophobicity plot (Fig. 8). All extracellular cysteines including the two cysteine residues in the presumed signal peptide are conserved, as are all potential, extracellular

N-glycosylation sites. A number of the amino acids which were not identical between the human kidney and rat bone PTH/PTHr receptors were found to be conserved between the human and the opposum receptors. These conserved amino 5 acids include an Arg to Leu at 51, an Arg to Trp at 58, an Arg to His at 262, an Asp to His at 358, an Ile to Thr at 422, and a Thr to Leu at 427.

Biological Characterization

- Functional characterization of the biological 10 properties of the opossum and rat PTH/PTHrP receptors was performed in transiently transfected COS cells by a radioreceptor assay technique using both 125 I-PTHrP and 125I-NlePTH as radioligands, and by bioassays that measure ligand-stimulated cAMP accumulation, increase in
- 15 intracellular free calcium, and stimulation of inositol phosphate metabolism, by the methods cited above.
- Fig. 9 demonstrates that COS cells expressing OK-H bind 125I-PTHrP. These data also demonstrate that binding of PTHrP is inhibited when intact PTH (1-34) or 20 PTH anlogues which are shortened at their amino terminus (i.e. the 3-34 and 7-34 analogues, which contain Nle substitutions for methionine at positions 8 and 18 and a tyrosine substitution for phenylalanine at position 34) are used as competitors for binding. Similarly, binding 25 of 125 I-NlePTH to COS cells expressing OK-H was inhibited when PTHrP or PTHrP fragments were used as competitors. These data indicate that PTH and PTHrP both bind to the receptor encoded by OK-H.
- Fig. 10 demonstrates that COS cells expressing OK-30 H increase their concentration of intracellular free calcium when exposed to NlePTH, but to a smaller extent (mean =
- 39 nm), or not at all, when compared to COS cells expressing OK-O or R15B receptors (Fig. 12 and Fig. 14) 35 and stimulated with NlePTH. Unlike COS cells expressing

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OK-O or R15B, COS cells expressing OK-H do not show a detectable increase in metabolism of inositol phosphate when stimulated with NlePTH (Fig. 15).

Fig. 11 demonstrates that COS cells expressing OK5 O bind ¹²⁵I-PTHrP. These data also demonstrate that
binding of PTHrP is inhibited when intact PTH (1-34) or
PTH analogues which are shortened at their amino terminus
(i.e. the 3-34 and 7-34 analogues, which contain Nle
substitutions for methionine at positions 8 and 18 and a
10 tyrosine substitution for phenylalanine at position 34)
are used as competitors for binding. Similarly, binding
of ¹²⁵I-NlePTH to COS cells expressing OK-H was inhibited
when PTHrP or PTHrP fragments were used as competitors.
These data indicate that PTH and PTHrP both bind to the
15 receptor encoded by OK-O.

Fig. 12 demonstrates that COS cells expressing OK-O increase their concentration of intracellular free calcium and their rate of inositol phosphate metabolism after stimulation with NlePTH and PTHrP (Fig. 15).

Fig. 13 demonstrates that COS cells expressing R15B bind ¹²⁵I-PTHrP. These data also demonstrate that binding of PTHrP is inhibited when intact PTH (1-34) or PTH anlogues which are shortened at their amino terminus (i.e. the 3-34 and 7-34 analogues, which contain Nle substitutions for methionine at positions 8 and 18 and a tyrosine substitution for phenylalanine at position 34) are used as competitors for binding. Similarly, binding of ¹²⁵I-NlePTH to COS cells expressing OK-H was inhibited when PTHrP or PTHrP fragments were used as competitors.

30 These data indicate that PTH and PTHrP both bind to the receptor encoded by R15B.

Fig. 14 demonstrates that COS cells expressing R15B increase their concentration of intracellular calcium to an extent similar to stimulated COS cells expressing OK-O.

Fig. 15 demonstrates that COS cells expressing R15B or OK-O increase their rate of phosphatidyl inositol hydrolysis, as evidenced by the rapid increase in inositol trisphosphate (IP3) and inositol bisphosphate 5 (IP2) accumulation after stimulation of the cells with NlePTH or PTHrP. Conversely, COS cells expressing OK-H did not show any detectable increase in inositol trisphosphate and inositol bisphosphate accumulation after stimulation with NlePTH or PTHrP. These data 10 suggest that the PTH receptor encoded by R15B and OK-O is coupled to phospholipase C, presumably through Gp. the only difference between OK-O and OK-H is in the cytoplasmic C-terminal tail, these data strongly suggest that the C-terminus of the PTH receptor encoded by OK-O 15 and R15B is involved in the activation of phospholipase

Fig. 16 demonstrates that COS cells expressing R15B and OK-H increase cAMP accumulation after stimulation with NlePTH. Similar results were obtained 20 in COS cells expressing OK-O. No cAMP stimulation was detected in COS cells transfected with the cDM8 vector alone. These data suggest that PTH receptor coupling to adenylate cyclase does not require the full length Cterminal cytoplasmic tail of the receptor.

25

These data demonstrate that all three PTH/PTHrP receptors cloned from both OK and ROS cell cDNA libraries bind the amino-terminal ligands of both peptides equivalently. Activation of all these receptors by ligand stimulates adenylate cyclase (as measured by 30 increased intracellular cAMP), presumably through activation of one class of guanine nucleotide binding proteins (G-proteins). G-proteins have a trimeric peptide structure in which one of the subunits, alpha, is distinct, and the other two, beta and gamma, are 35 identical or highly homologous. One of these G-proteins

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(G_c) contains G-alpha-"stimulatory" (G-alpha-s) which is involved in the activation of adenylate cyclase.

Binding of ligand to OK-O and R15B, but not to OK-H, also increases intracellular free calcium and 5 stimulates metabolism of inositol phosphate. These properties strongly suggest that activation of both OK-O and R15B receptors by ligand results in stimulation of a second intracellular effector, phospholipase C. coupling mechanism between these activated receptors and 10 phospholipase C is likely to be a G-protein which is distinct from G. In contrast, the properties of the activated OK-H receptor which is truncated at the carboxy terminus, suggest that it may not activate phospholipase C, or that it activates phospholipase C inefficiently.

15

The biochemical role of the carboxy-terminal tail of the PTH/PTHrP receptor was further investigated by the construction of a carboxy-terminally-truncated rat receptor, R480, by standard PCR technology using R15B as a template and an upstream primer containing a stop codon 20 inserted at position 481. Briefly, the upstream primer was a synthetic oligonucleotide based on nucleotides 1494-1513 of the rat cDNA sequence (see Fig. 3; SEQ ID NO.: 3) to which a stop codon and an XbaI cloning site were added. Thirty PCR cycles were carried out, each 25 cycle consisting of 1 min at 92°C for denaturation, 1 min at 60°C for annealing, and 1 min at 72°C for extension. The product was cut with NsiI and XbaI and purified by gel electrophoresis. R15B was sequentially digested with XbaI and NsiI, and the purified PCR product was then 30 ligated into the XbaI-NsiI cut R15B vector. The resulting plasmid, R480, was amplified in bacteria and sequenced.

R480 encodes 480 amino acids that are identical to those in the 591 amino acids receptor. This truncated 35 cDNA was expressed in COS-7 cells (transient expression)

and in CHO cells (stable expression). Both COS-7 and CHO cells expressing the truncated receptor, R480, and the wild type receptor, RB, bind PTH(1-34) with equivalent affinities. When activated, R480 stimulates cAMP 5 accumulation in COS7 and CHO cells as efficiently as does the wild type receptor. In contrast to the wild type receptor, R480 did not mediate any increase in [Ca2+]i when stimulated by PTH in either the COS-7 cells or the CHO cells. These data indicate that the molecular 10 requirements for activation of phospholipase C and adenylate cyclase by PTH/PTHrP receptor are distinct from each other, and point to a major role of the carboxyterminal tail of the PTH/PTHrP receptor in coupling to phospholipase C but not to adenylate cyclase. Of course, 15 it is also possible that activated PTH/PTHrP receptors may activate additional G-proteins and/or intracelluar effector molecules.

Analysis of COS-7 cells transfected with the cloned human PTH/PTHrP receptor demonstrated that 20 radiolabelled PTH(1-34) and PTHrP(1-36) (~200,000 cpm) bound to the expressed receptors with similar efficiency (specific binding: $10.1 \pm 3.7\%$ and $7.6\pm6.0\%$, respectively) to that observed for COS-7 cells expressing R15B (specific binding: 8.1+3.5% and 7.1+4.1%, 25 respectively). The expressed human PTH/PTHrP receptors bound PTH(1-34) with 2-fold higher apparent Kd than did the rat bone PTH/PTHrP receptor: ~5 nM versus ~10 nM (Fig. 17). However, despite their high degree of amino acid homology, the two receptors showed significant 30 differences in affinity for PTH(3-34) and PTH(7-34). PTHrP(1-36) displayed a 2- to 4-fold lower affinity for the human PTH/PTHrP receptor than for the rat receptor (~35 nM for HKrk versus ~10 nM for R15B) which appeared more pronounced when PTHrP(1-36) was used as radioligand. 35 The affinities for PTH(3-34) and PTH(7-34) were 7- and

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35-fold higher with the expressed HKrK than with R15B (~7 nM versus ~45 nM for PTH(3-34), respectively; ~60 nM versus ~2000 nM for PTH(7-34), respectively). In COS-7 cells expressing either receptor, both PTH(1-34) and PTHrP(1-36) stimulated the increase in intracellular free calcium and cAMP accumulation to the same extent (Fig. 18).

Relationship of PTH/PTHrP receptors

The amino acid sequence of the human PTH/PTHrP 10 receptor displays a very high degree of conservation compared to the bone PTH/PTHrP receptor from rat, a eutherian mammal, while its sequence identity with the PTH/PTHrP receptor with the opossum, a marsupial mammal, is less marked. Like the opossum kidney and the rat bone 15 receptor, the human kidney receptor induces an increase in both intra-cellular cAMP and intracellular free calcium when challenged with either PTH or PTHrP. Despite the high degree of homology between the human PTH/PTHrP receptor and the opossum and rat homologs, the 20 transiently expressed human receptor has some functional characteristics that are distinct from those of the rat bone receptor. These include a slightly higher affinity for PTH(1-34) and a significantly descreased affinity for PTHrP(1-36). Higher affinities were observed for PTH(3-25 34) and in particular for PTH(7-34), the affinity of which for the human receptor was about 35-fold higher in comparison to the rat bone receptor. These findings may have significant implications for the future development of PTH/PTHrP analogues, since they predict that species-30 specific tissues would be the appropriate tissues for testing the potency of antagonists (and agonists) in

Relationship of PTH/PTHrP receptors to other receptors

The biochemical properties of PTH and PTHrP 35 receptors suggest that they are members of the class of

vitro.

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membrane receptor molecules known as G-protein-linked membrane receptors. The structural features of well-characterized G-protein receptors indicate that they all have at least seven regions of several consecutive bydrophobic amino acids, each of which regions is of sufficient length to span the plasma membrane.

One subfamily of G-protein-linked membrane receptors, termed the glycopeptide receptor subfamily, includes receptors that bind and are activated by 10 glycopeptide hormones (thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone, and chorionic gonadotropin). All of these receptors are characterized by (1) extensive putative amino-terminal extracellular domains (greater than 300 amino acids) that 15 are thought to contain some or all of the ligand-binding domains, and (2) considerable amino-acid homology, particularly in the seven putative transmembrane domains. A second subfamily, termed the adrenergic/muscarinic subfamily, includes receptors that are activated by small 20 ligands, such as the catecholoamines, neuromuscular transmitters, and retinol. These receptors are all characterized by relatively short (25-75 amino acids) putative amino-terminal extracellular domains, as well as considerable amino acid homology, particularly in the 25 seven putative transmembrane domains. Activation of these receptors by their ligands appears to involve at least several of the multiple transmembrane domains, and does not appear to involve the amino-terminal portion of the receptors.

30 Several structural characteristics which can be deduced from the predicted amino acid sequence of the rat PTH/PTHrP receptor (Fig. 3) indicate that the PTH/PTHrP is a G-protein-linked receptor. The amino terminus shows characteristic features of a signal peptide, including a hydrophobic domain and the presence of three consecutive

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leucine residues. This amino acid stretch of 20-28 amino acids may serve as a leader sequence, similar to the amino terminus preceding the extracellular domains of other glycoprotein receptors. There is also a cluster of seven hydrophobic segments which represent putative membrane-spanning domains (Fig. 19).

The predicted amino acid sequences of the opossum kidney, rat bone and human kidney PTH/PTHrp receptors indicate that they do not fit comfortably into either of 10 these G-protein linked receptor subfamilies. Overall homology of the rat and human PTH/PTHrP receptors with the glycopeptide receptor and adrenergic/muscarinic subfamilies is approximately 10 to 20%, with a somewhat higher degree of homology within the transmembrane 15 domains. The latter is to be expected because of the limited menu of hydrophobic amino acids that could occur Twenty percent homology is far less in those regions. than that found among the receptors generally accepted to be members of each of these subfamilies. Additionally, 20 there are no portions of these sequences that have what could be characterized as intense homology (i.e., exactly matching amino acid sequences), even over limited regions.

Recent comparison with the newly characterized

25 secretin and calcitonin receptors (Ishihara et al., EMBO

J 10:1635, 1991; Lin et al., Science 254:1022, 1991) has
revealed between 30 and 40% identity between these
receptors and the PTH/PTHrP receptor. Although the
PTH/PTHrP receptor is more than 100 amino acids longer

30 than the calcitonin receptor, there is an ~32% identify
between the amino acid sequences of the opossum kidney
PTH/PTHrP receptor (SEQ ID NO NO.:2) and porcine kidney
calcitonin receptor (GenBank accession no. M74420). A
stretch of 17 out of 18 amino acids in the putative

35 transmembrane domain VII are identical. Also, two out of

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four N-linked glycoslyation sites and the position of seven out of eight potentially extracellular cysteines are conserved. Major differences between the two receptors appear to lie in their NH,-terminal and COOH-5 terminal domains. Comparison of amino acid sequences of the rat secretin receptor (GenBank accession no. X59132) and the human PTH/PTHrP receptor indicates that there is a 43% identity between these two receptors, with a stretch of 21 out of 25 amino acids of the putative 10 transmembrane domain VII being identical. The similarity between the PTH/PTHrP, calcitonin and secretin receptors suggests that they represent a new family of seven transmembrane-spanning G protein-coupled receptors that activate adenylate cyclase. Given the amino acid 15 sequences of these receptors, those skilled in the art would be able to compare these sequences for regions of identity which would be useful in the design of nucleic acid probes which could then be used for the identification and isolation of other receptors which 20 would belong to this family.

Deposit of Clones

Under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure, the 25 cDNA expression plasmids R15B, OK-O, and OK-H; the phage HPG1; and a plasmid (termed 8A6) containing part of the human clone have been deposited with the American Type Culture Collection (ATCC), where they bear the respective accession numbers ATCC No. 68571, 68572, 68573, 40998 and 68570. Applicants' assignee, The General Hospital Corporation, represents that the ATCC is a depository affording permanence of the deposits and ready accessibility thereto by the public if a patent is granted. All restrictions on the availability to the

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public of the material so deposited will be irrevocably removed upon the granting of a patent. The material will be available during the pendency of the patent application to one determined by the Commissioner to be 5 entitled thereto under 37 CFR 1.14 and 35 U.S.C. 122. The deposited material will be maintained with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposited 10 plasmid, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of the patent, whichever period is longer. Applicants' assignee acknowledges its responsibility to replace the deposits should the 15 depository be unable to furnish a sample when requested due to the condition of the deposit.

POLYPEPTIDES

Polypeptides according to the invention include the opossum and rat and human parathyroid hormone receptors as shown in Figs. 1-3 and 6, respectively, and any other naturally-occurring receptor which can be produced by methods analogous to those used to clone and express these receptors, or by methods utilizing as a probe all or part of one of the sequences described herein. In addition, any analog or fragment of a PTH receptor capable of binding to a parathyroid hormone or a parathyroid hormone-related protein is within the invention.

Specific receptor analogs of interest include

30 full-length or partial receptor proteins having an amino acid sequence which differs only by conservative amino acid substitutions: for example, substitution of one amino acid for another of the same class (e.g., valine for glycine; arginine for lysine, etc.), or by one or

35 more non-conservative amino-acid substitutions,

deletions, or insertions located at positions which do not destroy the receptor's ability to bind to parathyroid hormone or parathyroid hormone-related protein.

Specific receptor fragments of particular interest include, but are not limited to, portions of the receptor deduced to be extracellular from the primary amino acid sequence, using a hydrophobicity/hydrophilicity calculation such as the Chou-Fasman method (see, e.g., Chou and Fasman, Ann. Rev. Biochem. 47:251, 1978).

- 10 Hydrophilic domains, particularly ones surrounded by hydrophobic stretches (e.g., transmembrane domains) of at least 10 amino acids, present themselves as strong candidates for extracellular domains. Fig. 21 illustrates a predicted arrangement of extracellular,
- 15 intracellular, and transmembrane domains of one PTH receptor.

Examples of specific PTH receptor fragments include those with the following amino acid sequences (shown as standard single-letter symbols), derived from

20 the deduced amino acid sequence of the R15B clone: Extracellular domains:

RP-1: TNETREREVFDRLGMIYTVG (SEQ ID NO.: 5)

RP-2: VLYSGFTLDEAERLTEEEL (SEQ ID NO.: 6)

RP-3: VTFFLYFLATNYYWILVEG (SEQ ID NO.: 7)

25 RP-4: Y-RATLANTGCWDLSSGHKKWIIQVP (SEQ ID NO.: 8)

RP-5: PYTEVSGTLWQIQMHYEM (SEQ ID NO.: 9)

RP-6: DDVFTKEEQIFLLHRAQA (SEQ ID NO.: 10)

Intracellular domains:

RPi-7: FRRLHCTRNY (SEQ ID NO.: 11)

30 RPi-8: EKKYLWGFTL (SEQ ID NO.: 12)

RPi-9: VLATKLRETNAGRCDTRQQYRKLLK (SEQ ID NO.: 13)
These fragments were synthesized and purified by HPLC according to the method of Keutmann et al.,
(Endocrinology 117: 1230, 1984).

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EXPRESSION OF POLYPEPTIDES

Polypeptides according to the invention may be produced by expression from a recombinant nucleic acid having a sequence encoding part or all of a cell receptor 5 of the invention, using any appropriate expression system: e.g., transformation of a suitable host cell (either prokaryotic or eukaryotic) with the recombinant nucleic acid in a suitable expression vehicle (e.g., pcDNAI). The precise host cell used is not critical to 10 the invention; however, in the case wherein the polypeptides of the invention include all or part of the PTH/PTHrP receptor, the following host cells are preferred: COS cells, LLC-PK1 cells, OK cells, AtT20 cells, and CHO cells. The method of transfection and the 15 choice of expression vehicle will depend on the host system selected. Mammalian cell transfection methods are described, e.g., in Ausubel et al. (Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989); expression vehicles may be chosen from those discussed, 20 e.g., in Cloning Vectors: A Laboratory Manual (P.H. Pouwels et al., 1985, Supp. 1987). Stably transfected cells are produced via integration of receptor DNA into the host cell chromosomes. Suitable DNAs are inserted into pcDNA, pcDNAI-Neo, or another suitable plasmid, and 25 then cells are transfected with this plasmid with or without cotransfection with psV-2-Neo, or psV-2-DHFR by standard electroporation, calcium phosphate, and/or DEAE/Dextran techniques. Selection of transfected cells is performed using progressively increasing levels of 30 G418 (Geneticin, GIBCO), and if necessary, methotrexate.

DNA sequences encoding the polypeptides of the invention can also be expressed in a prokaryotic host cell. DNA encoding a cell receptor or receptor fragment is carried on a vector operably linked to control signals capable of effecting expression in the prokaryotic host.

If desired, the coding sequence may contain, at its 5' end, a sequence encoding any of the known signal sequences capable of effecting secretion of the expressed protein into the periplasmic space of the host cell, 5 thereby facilitating recovery of the protein and subsequent purification. Prokaryotes most frequently used are various strains of E. coli; however, other microbial strains may also be used. Plasmid vectors are used which contain replication origins, selectable 10 markers, and control sequences derived from a species compatible with the microbial host. For example, E. coli may be transformed using derivatives of pBR322, a plasmid constructed by Bolivar et al. (Gene 2: 95, 1977) using fragments derived from three naturally-occurring 15 plasmids, two isolated from species of Salmonella, and one isolated from E. coli. pBR322 contains genes from ampicillin and tetracycline resistance, and thus provides multiple selectable markers which can be either retained or destroyed in constructing the desired expression 20 vector. Commonly used prokaryotic control sequences (also referred to as "regulatory elements") are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences. Promoters commonly used to direct 25 protein expression include the beta-lactamase (penicillinase), the lactose (lac) (Chang et al., Nature 198: 1056, 1977) and the tryptophan (Trp) promoter systems (Goeddel et al., Nucl. Acids Res. 8: 4057, 1980) as well as the lambda-derived $P_{r_{\rm L}}$ promoter and N-gene 30 ribosome binding site (Simatake et al., Nature 292:128,

The nature of the cell receptor proteins of the invention is such that, upon expression within a cell, it is moved to the cellular membrane and partially through the membrane, so that part of it remains embedded in the

1981).

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membrane, part extends outside the cell, and part remains within the cell. Transformed cells bearing such embedded cell receptors may themselves be employed in the methods of the invention, or the receptor protein may be extracted from the membranes and purified.

Expression of peptide fragments lacking the hydrophobic portions of the protein responsible for anchoring the intact protein in the cellular membrane would not be expected to become embedded in the membrane; 10 whether they remain within the cell or are secreted into the extracellular medium depends upon whether or not a mechanism promoting secretion (e.g., a signal peptide) is included. If secreted, the polypeptide of the invention can be harvested from the medium; if not, the cells must be broken open and the desired polypeptide isolated from the entire contents of the cells. Specific examples of polypeptides which might be expressed include, without limitation:

- Amino-terminal portion comprising amino acids
 1-192, including the putative leader sequence, of the rat bone PTH/PTHrP receptor as shown in Fig. 3.
 - 2) Amino-terminal portion comprising amino acids 27-192, excluding the putative leader sequence, of the rat bone PTH/PTHrP receptor as shown in Fig. 3.
 - 25 3) The full-length PTH/PTHrP receptor from rat bone, as shown in Fig 3.
 - 4) RP-1 (as described above).
 - 5) RP-2 (as described above).

The polypeptide of the invention can be readily
purified using affinity chromatography. Antibodies to
these polypeptides, or the receptor specific ligands,
(e.g., the hormones PTH and PTHrP for the PTH/PTHrP
receptor) may be covalently coupled to a solid phase
support such as Sepharose 4 CNBr-activated sepharose
(Pharmacia), and used to separate the polypeptide of the

invention from any contaminating substances. Typically 1 mg of ligand or antibody will be incubated with CNBractivated sepharose at 4°C for 17-20 h (with shaking). The sepharose is rinsed with 1 M Tris HCL (pH8) to block excess active sites. The sepharose-PTH, sepharose-PTHrP, or sepharose-antibody is then incubated with the crude polypeptide in phosphate-buffered saline (pH 7.4) at 4°C for 2 h (with shaking). The sepharose is then typically packed in a column, thoroughly washed with PBS (typically 10 times the column volume), and eluted with dilute HCl in H₂O (pH 1.85). The eluate may then be concentrated by lyophylization and its purity checked, for example, by reverse phase HPLC.

ANTI-CELL RECEPTOR ANTIBODIES

Cell receptor or receptor fragments of the 15 invention may be used to generate antibodies by any conventional method well known to those skilled in the art, including those which generate polyclonal antibodies and those which generate monoclonal antibodies. 20 example, the deduced amino acid sequence of the PTH receptor reveals a protein structure that appears to have several transmembrane (i.e., hydrophobic) domains interspersed with presumably extracellular and intracellular regions (see Fig. 21) analogous to those 25 found in other G protein-linked receptors. This information can be used to guide the selection of regions of the receptor protein which would be likely to be exposed on the cell surface, and thus would be presented to antibodies in vivo. A short peptide representing one 30 or more of such regions may be synthesized (e.g., chemically or by recombinant DNA techniques) and used to immunize an animal (e.g., a rabbit or a mouse) to generate polyclonal or monoclonal antibodies. For example, certain of the peptides of the PTH/PTHrP 35 receptor listed above (RP-1, RP-5 and RP-6) have been

chemically synthesized using standard techniques and used to generate polyclonal antibodies in rabbits by the following procedure:

A preparation of a given peptide emulsified with 5 complete Freund's Adjuvant is injected intradermally into rabbits. Booster injections are emulsified in or complete adjuvant and injected at monthly intervals.

Antibody titer is assessed using either of two methods. First, serial dilutions of the antiserum in 1% 10 normal rabbit serum are incubated with 125I-labelled PTH/PTHrP receptor fragment by standard methods (e.g., see Segre et al., supra) for 24 h at 4° C. The bound 125 I-PTH/PTHrP receptor fragments are separated from unbound by addition of 100 μ l of second antibody (anti-15 rabbit IgG, Sigma) diluted 1:20 and 1 ml of 5% polyethylene glycol, followed by centrifugation at 2000 rpm for 30 min. at 4° C. The supernatant is removed and the pellet analyzed for radioactivity in a γ -counter. In the second method, cell lines expressing either native 20 (e.g., ROS 17/2.8, OK, SaOS-02 cells) or recombinant (COS cells or CHO cells transfected with R15B, OK-O or OK-H) PTH/PTHrP receptors are incubated with serially diluted antibody at 4°C, 20°C or 37°C for

1- 4 h. The cells are rinsed with PBS (x3) and incubated
25 for 2 h at 4°C with ¹²⁵I-labelled (NEN, Dupont) or FITClabelled (Sigma) second antibodies. After rinsing (x3
with PBS), the cells were either lysed with 0.1 M NaOH
and counted in γ-counter (if ¹²⁵I-labelled second antibody
was used) or fixed with 1% paraformaldehyde and examined
30 by fluorescent microscopy (if FITC-labelled second
antibody was used).

Another method for producing antibodies utilizes as antigen the intact cell receptor protein of the invention expressed on the surface of cells (e.g., 35 mammalian cells, such as COS cells, transfected with DNA

30

encoding the receptor). Such cells are prepared by standard techniques, e.g., by the DEAE-dextran transfection method, using a vector encoding and capable of directing high-level expression of the cell receptor. Such cells may be used to generate polyclonal or monoclonal antibodies. For example, monoclonal antibodies specific for the PTH/PTHrP receptor may be produced by the following procedure:

Intact COS cells expressing high levels of rat 10 recombinant PTH receptors on the cell surface are injected intraperitoneally (IP) into Balb-c mice (Charles River Laboratories, Willmington, MA). The mice are boosted every 4 weeks by IP injection, and are hyperimmunized by an intravenous (IV) booster 3 days 15 before fusion. Spleen cells from the mice are isolated and are fused by standard methods to myeloma cells. Hybridomas are selected in standard hypoxanthine/aminopterin/thymine (HAT) medium, according to standard methods. Hybridomas secreting antibodies 20 which recognize the PTH receptor are initially identified by screening with cell lines which naturally express abundant copies of the PTH-receptor per cell (such as ROS17/2.8 or OK cells), using standard immunological techniques. Those hybridomas which produce antibodies 25 capable of binding to the PTH receptor are cultured and subcloned. Secondary screening with radioreceptor and cAMP stimulation assays can then be performed to further characterize the monoclonal antibodies (see below). SCREENING FOR PTH RECEPTOR ANTAGONISTS AND AGONISTS

The polypeptides and antibodies of the invention and other compounds may be screened for PTH-competition and for antagonistic or agonistic properties using the assays described herein.

In one example, those antibodies that recognize the PTH receptor on the intact cells are screened for

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their ability to compete with PTH or PTHrP for binding to a PTH/PTHrP receptor. Cells expressing PTH receptor on the cell surface are incubated with the ¹²⁵I-PTH analog, ¹²⁵I-NlePTH or ¹²⁵I-PTHrP in the presence or absence of the polyclonal or monoclonal antibody to be tested, for 4 h at 15°C. The antibody used may be from crude antiserum, cell medium, or ascites, or in purified form. After incubation, the cells are rinsed with binding buffer (e.g., physiological saline), lysed, and quantitatively analyzed for radioactivity using a gammacounter. Antibodies that reduce binding of the PTH analog to the PTH receptor are classified as competitive; those which do not are noncompetitive.

Compounds, including antibodies and polypeptides, 15 may be screened for their agonistic or antagonistic properties using the cAMP accumulation, intracellular calcium, and/or inositol phosphate assays described above. Cells expressing PTH receptor on the cell surface are incubated with PTH, PTH-receptor antibody, or a 20 combination of both, for 5 - 60 minutes at 37°C, in the presence of 2 mM IBMX (3-isobutyl-1-methyl-xanthine, Sigma, St. Louis, MO). Cyclic AMP accumulation is measured by specific radio-immunoassay, as described above. A compound that competes with PTH for binding to 25 the PTH receptor, and that inhibits the effect of PTH on cAMP accumulation, is considered a competitive PTH antagonist. Conversely, a compound that does not compete for PTH binding to the PTH receptor, but which still prevents PTH activation of cAMP accumulation (presumably 30 by blocking the receptor activation site) is considered a non-competitive antagonist. A compound that competes with PTH for binding to the PTH receptor, and which stimulates cAMP accumulation in the presence or absence of PTH, is a competitive agonist. A compound that does 35 not compete with PTH for binding to the PTH receptor but

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which is still capable of stimulating cAMP accumulation in the presence or absence of PTH, or which stimulates a higher accumulation than that observed by PTH alone, would be considered a non-competitive agonist.

.5 <u>USE</u>

The polypeptides, antibodies, and other compounds of the invention are useful for the diagnosis, classification, prognosis, and/or treatment of disorders which may be characterized as related to the interaction 10 between a cell receptor of the invention and its specific ligand. For example, some forms of hypercalcemia and hypocalcemia are related to the interaction between PTH and PTHrP and the PTH/PTHrP receptor(s). Hypercalcemia is an condition in which there is an abnormal elevation 15 in serum calcium level; it is often associated with other diseases, including hyperparathyroidism, osteoporosis, carcinomas of the breast, lung and prostrate, epidermoid cancers of the head and neck of the esophagus, multiple myeloma, and hypernephroma. Hypocalcemia, a condition in 20 which the serum calcium level is abnormally low, may result from a deficiency of effective PTH, e.g., following thyroid surgery.

In a first example, the compounds of the invention are used to manufacture diagnostic agents which are used as diagnostic tools to diagnose hypercalcemia and to distinguish between hypercalcemic conditions, i.e., to differentiate hypercalcemia mediated by PTH or PTHrP (e.g., hyperparathyroidism and humoral hypercalcemia of malignancy), from hypercalcemia associated with diseases which do not involve these factors (e.g., local osteolytic hypercalcemia mediated by the presence of metastatic tumor cells in direct contact with bone, and certain rare types of malignancy-related hypercalcemias mediated by an increase of humoral factors, such as osteoclast activating factor (interleukin), lymphotoxin,

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calcitriol, type E prostaglandins, and vitamin D-like sterols).

In one method of diagnosis, serum total and/or ionized calcium levels are measured by standard 5 techniques before and after the administration of the PTH or PTHrP antagonists of the invention. PTH or PTHrP related hypercalcemias would be detectable as a decrease in serum calcium levels following administration of the antagonist of the invention. In contrast, for 10 hypercalcemic conditions mediated by factors other than PTH or PTHrP, the serum calcium levels would remain unchanged even after administration of the antagonist.

Another diagnostic application of the invention permits measurement of the level of PTH or PTHrP in a 15 biological sample in order to diagnose PTH or PTHrP related tumors, e.g., tumors which are associated with humoral hypercalcemia of malignancy, and for monitoring . the levels of PTH or PTHrP during cancer therapy. method involves assaying binding of the recombinant 20 parathyroid hormone receptor of the invention to PTH or PTHrP present in a tissue sample, using the binding assay described herein. The level of binding may be determined directly (e.g., by using radioactively labelled PTH receptor, and assaying the radioactivity bound to 25 endogenous PTH). Alternatively, binding of PTH receptor to the sample (e.g., a tissue section) may be followed by staining of the tissue sections with an antibody specific for the PTH receptor, using standard immunological techniques (Chin et al., Hybridoma 5:339, 1986).

In a third diagnostic approach, one could stably transfect cell lines (by the methods described in Ausubel et al., Current Protocols in Molecular Biology, Wiley Publishers, New York, 1987) with a PTH receptor gene linked to an appropriate promoter (e.g., the 35 metallothionine promoter). Alternatively, the PTH/PTHrP

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receptor could be expressed from a eukaryotic vector, i.e., pcDNAI, and cotransfected with a mutant DHFR gene that will allow further gene amplification via methotrexate selection (Simonsen et al., Proc. Natl. 5 Acad. Sci., 80:2495-2499, 1983). Such high-level expression of the gene produces an immortal cell line which is oversensitive to PTH or PTHrP. Such cells provide a particularly useful tool for detecting serum blood levels of PTH or PTHrP. Such a cell line may be 10 used for diagnosis of conditions involving elevated PTH or PTHrP levels (e.g., those described above) or for conditions involving unusually low levels of PTH or PTHrP (e.g., those described above). Such a cell line is also useful for monitoring the regression or increase of PTH 15 or PTHrP levels during therapy for hypercalcemia or hypocalcemia, respectively.

A patient who is suspected of being hypercalcemic may be treated using the compounds of the invention. Rapid intervention is important because symptoms may 20 appear abruptly and, unless reversed, can be fatal. one application, serum calcium levels are stabilized by an immediate course of treatment which includes antagonists of PTH or PTHrP. Such antagonists include the compounds of the invention which have been determined 25 (by the assays described herein) to interfere with PTH receptor-mediated cell activation. To administer the antagonist, the appropriate antibody or peptide (is used in the manufacture of a medicament, generally by being formulated in an appropriate carrier such as 30 physiological saline, and administered intravenously, at a dosage that provides adequate competition for PTH or PTHrP binding to the PTH receptor (e.g., a dosage sufficient to lower the serum calcium level to below 10 mg/dl). Typical dosage would be 1 ng to 10 mg of the 35 antibody or peptide per kg body weight per day.

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Treatment may be repeated as necessary for long term maintenance of acceptable calcium levels (i.e., levels < 10.1 mg/dl). This may be necessary for acute treatment of an underlying disease condition triggering 5 hypercalcemia; or it may used, e.g., for chronic treatment of conditions such as osteoporosis.

In another application, the compounds of the invention which have been characterized, according to the methods of the invention, to be agonists are used

10 therapeutically to treat hypocalcemia: e.g., that resulting from the partial or complete surgical removal of the parathyroid glands. Agonists may be formulated in a suitable carrier (e.g., physiological saline) and are preferably administered intravenously in a dosage that

15 causes a rise in serum calcium to an acceptable level (i.e., approximately 8 mg/dl). A useful dosage range would be 1 ng to 10 mg of the agonist per kg body weight per day. Treatment may be repeated as necessary to maintain suitable serum calcium levels; long term

20 treatment may be necessary for patients who have undergone parathyroid gland removal.

The nucleic acids of the invention may also be used therapeutically. Oligonucleotides which are antisense to PTH receptor mRNA (or nucleic acid constructs which express RNA that is antisense to PTH receptor mRNA) may be utilized as an anticancer therapy. This approach is useful, e.g., for hypercalcemias resulting from a genomic rearrangement or amplification which increases the amount or activity of PTH receptor, PTH or PTHrP. The method would involve introduction of the antisense oligonucleotide into the tumor cells in vivo. The antisense strand hybridizes with endogenous PTH receptor mRNA, interfering with translation of the protein, thereby reducing production of PTH receptor in such cells, and reducing PTH/PTHrP-associated neoplastic

growth. Methods for antisense design and introduction into host cells are described, for example, in Weinberg et al., U.S. Patent No. 4,740,463, herein incorporated by reference. The biochemical characterization of the OK-5 H, OK-O and R15B PTH/PTHrP receptors of the invention demonstrate that the two transduction pathways now known to be triggered by the interaction of PTH with its receptor are distinct and may be separated. predicted amino acid sequences of these receptors 10 indicate that OK-H, which does not appear to activate inositol phosphate metabolism to any detectable degree, is 70 amino acids shorter at the carboxy-terminus than OK-O or R15B. By using the sequences of the invention and the information disclosed herein, one could clone and 15 then alter (e.g. by site-directed mutagenesis) PTH/PTHrP receptor genes from any species to generate PTH/PTHrP receptors which do not activate phospholipase C. could potentially allow the separation of different PTHmediated actions, including bone resorption and bone 20 formation, and could of great importance for the treatment of various bone disorders such as osteoporosis.

Nucleic acids of the invention which encode a PTH receptor may also be linked to a selected tissue-specific promoter and/or enhancer and the resultant hybrid gene
introduced, by standard methods (e.g., as described by Leder et al., U.S. Patent No. 4,736,866, herein incorporated by reference), into an animal embryo at an early developmental stage (e.g., the fertilized oocyte stage), to produce a transgenic animal which expresses elevated levels of PTH receptor in selected tissues (e.g., the osteo calcin promoter for bone). Such promoters are used to direct tissue-specific expression of the PTH receptor in the transgenic animal. The form of PTH receptor utilized can be one which encodes a PTH receptor similar to that of the animal species used, or

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it can encode the PTH receptor homolog of a different species. In one particular example, transgenic chickens are engineered to express the PTH receptor from a promoter which directs high-level expression in chicken oviducts. Such an animal is expected to produce eggs with higher calcium content, and thus harder shells.

Other Embodiments

Other embodiments are within the following claims. For example, the nucleic acid of the invention includes genes or cDNAs or RNAs originally isolated from any vertebrate species, including birds or mammals such as marsupials, rodents, or humans. The high degree of homology demonstrated for the PTH receptors from such diverse species as opossum, rat, and human indicates that the methods of isolating PTH receptors disclosed herein will be broadly applicable to the isolation of related cell receptors from a wide variety of species.

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COMPUTER SUBMISSION OF DNA AND AMINO ACID SEQUENCES

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION:

PARATHYROID HORMONE RECEPTOR AND DNA

ENCODING SAME

(iii) NUMBER OF SEQUENCES:

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U.S.A.

(F) ZIP:

02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE:

(D) SOFTWARE:

3.5" Diskette, 1.44 Mb storage

(B) COMPUTER:

IBM PS/2 Model 502 or 55SX

(C) OPERATING SYSTEM:

IBM P.C. DOS (Version 3.30) WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:

07/681,702

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- 51 -

	(0) TE	LEX:							20	0154					
(2)	INFO	RMAT	ION	FOR	SEQU	ENCE	IDE	NTIF	ICAT	ION	NUMB	ER:	1	:		
	(i) SE	QUEN	CE C	HARA	CTER	ISTI	cs:								
									(B) (C)	TYPE STRA	TH:1 :nuc NDED LOGY	leic NESS	:dou			
	(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO: 1: GGGCACAGC CACCCTGTTG GTAGTCCAGG GGCCAGCCCA CTGAGCTGGC ATATCAGCTG 60															
										ATG	GGA	GCG	CCC	CGG	AGCTG ATC Ile 5	60 115
TCG Ser	CAC His	AGC Ser	CTT Leu 10	GCC Ala	TTG Leu	CTC Leu	CTC Leu	TGC Cys 15	TGC Cys	TCC Ser	GTG Val	CTC Leu	AGC Ser 20	TCC Ser	GTC Val	163
											AAG Lys					211
ATT Ile	CTT Leu 40	CTG Leu	CGC Arg	AAT Asn	GCC Ala	CAG Gln 45	GCC Ala	CAG Gln	TGT Cys	GAG Glu	CAG Gln 50	CGC Arg	CTG Leu	AAA Lys	GAG Glu	259
GTC Val 55	CTC Leu	AGG Arg	GTC Val	CCT Pro	GAA Glu 60	CTT Leu	GCT Ala	GAA Glu	TCT Ser	GCC Ala 65	AAA Lys	GAC Asp	TGG Trp	ATG Met	TCA Ser 70	307
AGG Arg	TCT Ser	GCA Ala	AAG Lys	Thr	AAG Lyв 75	AAG Lys	GAG Glu	AAA Lys	Pro	GCA Ala 30	GAA Glu	AAG Lys	CTT Leu	Tyr	CCC Pro 35	355
CAG Gln	GCA Ala	GAG Glu	GAG Glu 90	Ser	AGG Arg	GAA Glu	GTT Val	TCT Ser	Asp	AGG Arg	AGC Ser	CGG Arg	Leu	CAG Gln DO	GAT Asp	403
											TGC Cys					451

GTG CCC GGC AAG GTG GTG GCC GTG CCC TGC CCC GAC TAC TTC TAC GAC 499

547

Val Pro Gly Lys Val Val Ala Val Pro Cys Pro Asp Tyr Phe Tyr Asp

TTC AAC CAC AAA GGC CGA GCC TAT CGG CGC TGT GAC AGC AAT GGC AGC

Phe Asn His Lys Gly Arg Ala Tyr Arg Arg Cys Asp Ser Asn Gly Ser

125

140

120

135

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TGG Trp	GAG Glu	CTG Leu	GTG Val	CCT Pro 15	Gly	AAC Asn	AAC Asn	CGG Arg	ACA Thr 16	Trp	GCG Ala	AAT Asn	TAC Tyr	AGC Ser	Glu	595
TGT Cyb	GTC Val	AAG Lys	TTT Phe 17	Leu	ACC Thr	AAC Asn	GAG Glu	ACC Thr	Arg	GAA Glu	CGG Arg	GAA Glu	Val	TTT Phe 30	GAT Asp	643
CGC Arg	CTC Leu	GGA Gly 185	ATG Met	ATC Ile	TAC Tyr	ACT Thr	GTG Val 190	GGC	TAC Tyr	TCC Ser	ATC Ile	TCT Ser 195	CTG Leu	GGC Gly	TCC Ser	691
CTC Leu	ACT Thr 200	GTG Val	GCT Ala	GTG Val	Leu CTG	ATT Ile 205	CTG Leu	GGT Gly	TAC Tyr	TTT Phe	AGG Arg 210	AGG Arg	TTA Leu	CAT His	TGC Cys	739
ACC Thr 215	CGA Arg	AAC Asn	TAC Tyr	ATT Ile	CAC His 220	ATG Met	CAT His	CTC Leu	TTC Phe	GTG Val 225	TCC Ser	TTT Phe	ATG Met	CTC Leu	CGG Arg 230	787
GCT Ala	GTA Val	AGC Ser	ATC Ile	TTC Phe 23	Ile	AAG Lys	GAT Asp	GCT Ala	Val	CTC Leu 40	TAC Tyr	TCG Ser	GGG Gly	Val	TCC Ser 45	835
ACA Thr	GAT Asp	GAA Glu	ATC Ile 250	GAG Glu	CGC Arg	ATC Ile	ACC Thr	GAG Glu 255	GAG Glu	GAG Glu	CTG Leu	AGG Arg	GCC Ala 260	TTC Phe	ACA Thr	883
GAG Glu	CCT Pro	CCC Pro 265	CCT Pro	GCT Ala	GAC	AAG Lys	GCG Ala 270	GGT Gly	TTT Phe	GTG Val	GGC Gly	TGC Cys 275	AGA Arg	GTG Val	GCG Ala	931
GTA Val	ACC Thr 280	GTC Val	TTC Phe	CTT Leu	TAC Tyr	TTC Phe 285	CTG Leu	ACC Thr	ACC Thr	AAC Asn	TAC Tyr 290	TAC Tyr	TGG Trp	ATC Ile	CTG Leu	979
GTG Val 295	GAA Glu	GGC Gly	CTC Leu	TAC Tyr	CTT Leu 300	CAC His	AGC Ser	CTC	ATC Ile	TTC Phe 305	ATG Met	GCT Ala	TTT Phe	TTC Phe	TCT Ser 310	1027
GAG Glu	AAA Lys	AAG Lys	TAT Tyr	Leu	TGG Trp 15	GGT Gly	TTC Phe	ACA Thr	Leu	TTT Phe 20	GGC	TGG Trp	GGC Gly	Leu	CCT Pro 25	1075
GCC Ala	GTG Val	TTT Phe	GTC Val 330	Ala	GTG Val	TGG Trp	GTG Val	ACC Thr 335	Val	AGG Arg	GCT Ala	ACA Thr	CTG Leu 340	Ala	AAC Asn	1123
ACT Thr	GAG Glu	TGC Cys 345	Trp	GAC Asp	CTG Leu	AGT Ser	TCG Ser 350	Gly	AAT Asn	AAG Lys	AAA Lys	TGG Trp 355	Ile	ATA Ile	CAG Gln	1171

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														ATC Ile		1219
	360					365					370					
														GGG		1267
11e 375	Ile	Arg	Val	Leu	Ala 380	Thr	Lyв	Leu	Arg	G1u 385	Thr	Asn	Ala	Gly	Arg 390	
TGT	GAC	ACG	AGG	CAA	CAG	TAT	AGA	AAG	CTG	CTG	AAG	TCC	ACG	CTA	GTC	1315
Сув	Авр	Thr	Arg	Gln	Gln	Tyr	Arg	Lys	Leu	Leu	Lys	Ser	Thr	Leu		
				39	95				40	00				40)5	
														ACG		1363
Leu	Met	Pro			Gly	Val	His			Val	Phe	Met		Thr	Pro	
			4:	10				4:	15				4:	20		
TAC	ACA	GAA	GTA	TCA	GGG	ATT	CTT	TGG	CAA	GTC	CAA	ATG	CAC	TAT	GAA	1411
Tyr	Thr	Glu	Val	Ser	Gly	Ile	Leu	Trp	Gln	Val	Gln		His	Tyr	Glu	
		425					430					435				
														TAC		1459
Met	Leu	Phe	Asn	Ser	Phe		Gly	Phe	Phe	Val		Ile	Ile	Tyr	Сув	
	440					445					450					
TTC	TGC	AAT	GGA	GAG	GTA	CAA	GCA	GAG	ATC	AAG	AAG	TCA	TGG	AGC	CGA	1507
Phe	Сув	Asn	Gly	Glu		Gln	Ala	Glu	Ile		Lys	Ser	Trp	Ser		
455					460					465					470	
														AGC		1555
Trp	Thr	Leu	Ala			Phe	Lys	Arg			Arg	Ser	Gly	Ser		
				4	75				4	80				4.	B 5	
														AAT		1603
Thr	Tyr	Ser	_		Pro	Met	Val		His	Thr	Ser	Val		Asn	Val	
			490					495					500			
														CTCC	TGG	1652
Gly	Pro	_	_	Gly	Trp	Pro			Ser	Ala	Leu					
		505					510					515				
ccc	mca*		አርጥር	ררא א	ጥር ር	ር ርኔጥ	רצייר	ጆ ርጥ	ጥርሶሶ	ተርርር	ጥልጥ	ፈጋጥጋ	AGC	ATGG	TTCCAT	1712
															GTATCT	
															GGAGGA	
							דבדב									1862

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:	
(i) SEQUENCE CHARACTERISTICS:	-
(A) LENGTH: 1863 (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(xi) SEQUENCE DESCRIPTION: SEQUENCE ID NO: 2:	
TGGGCACAGC CACCCTGTTG GTAGTCCAGG GGCCAGCCCA CTGAGCTGGC ATATCAGCTG	60
GTGGCCCCGT TGGACTCGGC CCTAGGGAAC GGCGGCG ATG GGA GCG CCC CGG ATC Met Gly Ala Pro Arg Ile 1 5	115
TCG CAC AGC CTT GCC TTG CTC CTC TGC TGC TCC GTG CTC AGC TCC GTC	163
Ser His Ser Leu Ala Leu Leu Cys Cys Ser Val Leu Ser Ser Val 10 15 20	
TAC GCA CTG GTG GAT GCC GAT GAT GTC ATA ACG AAG GAG GAG CAG ATC	211
Tyr Ala Leu Val Asp Ala Asp Asp Val Ile Thr Lys Glu Glu Gln Ile 25 30 35	
ATT CTT CTG CGC AAT GCC CAG GCC CAG TGT GAG CAG CGC CTG AAA GAG	259
Ile Leu Leu Arg Asn Ala Gln Ala Gln Cys Glu Gln Arg Leu Lys Glu 40 45 50	
GTC CTC AGG GTC CCT GAA CTT GCT GAA TCT GCC AAA GAC TGG ATG TCA	307
Val Leu Arg Val Pro Glu Leu Ala Glu Ser Ala Lys Asp Trp Met Ser 55 60 65 70	
AGG TCT GCA AAG ACA AAG AAG GAG AAA CCT GCA GAA AAG CTT TAT CCC	355
Arg Ser Ala Lys Thr Lys Lys Glu Lys Pro Ala Glu Lys Leu Tyr Pro 75 80 85	
CAG GCA GAG GAG TCC AGG GAA GTT TCT GAC AGG AGC CGG CTG CAG GAT Gln Ala Glu Glu Ser Arg Glu Val Ser Asp Arg Ser Arg Leu Gln Asp	403
90 95 100	
GGC TTC TGC CTA CCT GAG TGG GAC AAC ATT GTG TGC TGG CCT GCT GGA	451
Gly Phe Cys Leu Pro Glu Trp Asp Asn Ile Val Cys Trp Pro Ala Gly 105 110 115	
	499
GTG CCC GGC AAG GTG GTG GCC GTG CCC TGC CCC GAC TAC TTC TAC GAC	

Val Pro Gly Lys Val Val Ala Val Pro Cys Pro Asp Tyr Phe Tyr Asp

125

120

130

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							TAT Tyr									547
					Gly		AAC Asn			Trp					Glu	595
							GAG Glu									643
							GTG Val 190									691
							CTG Leu									739
							CAT His									787
							GAT Asp									835
				2:	35				24	40				24	15	
		Glu		GAG	CGC		ACC Thr		GAG	GAG				TTC	ACA	883
Thr	Asp	Glu CCC	Ile 250 CCT	GAG Glu GCT	CGC Arg	Ile		Glu 255 GGT	GAG Glu TTT	GAG Glu GTG	Leu	Arg TGC	Ala 260 AGA	TTC Phe GTG	ACA Thr	883 931
Thr GAG Glu GTA	ASP CCT Pro	Glu CCC Pro 265	Ile 250 CCT Pro	GAG Glu GCT Ala	CGC Arg GAC Asp	Ile AAG Lys	Thr GCG Ala	Glu 255 GGT Gly	GAG Glu TTT Phe	GAG Glu GTG Val	GGC Gly	TGC Cys 275	Ala 260 AGA Arg	TTC Phe GTG Val	ACA Thr GCG Ala	
Thr GAG Glu GTA Val	CCT Pro ACC Thr 280	CCC Pro 265 GTC Val	Ile 250 CCT Pro TTC Phe	GAG Glu GCT Ala CTT Leu	CGC Arg GAC Asp TAC Tyr	AAG Lys TTC Phe 285	Thr GCG Ala 270 CTG	Glu 255 GGT Gly ACC Thr	GAG Glu TTT Phe ACC Thr	GAG Glu GTG Val AAC ABn	GGC Gly TAC Tyr 290	TGC Cys 275 TAC Tyr	Ala 260 AGA Arg TGG Trp	TTC Phe GTG Val ATC Ile	ACA Thr GCG Ala CTG Leu	931
GAG Glu GTA Val GTG Val 295	CCT Pro ACC Thr 280 GAA Glu	CCC Pro 265 GTC Val GGC Gly	Ile 250 CCT Pro TTC Phe CTC Leu	GAG Glu GCT Ala CTT Leu TAC Tyr	CGC Arg GAC Asp TAC Tyr CTT Leu 300	AAG Lys TTC Phe 285 CAC His	GCG Ala 270 CTG Leu	Glu 255 GGT Gly ACC Thr CTC Leu	GAG Glu TTT Phe ACC Thr ATC Ile	GAG Glu GTG Val AAC Asn TTC Phe 305	GGC Gly TAC Tyr 290 ATG Met	TGC Cys 275 TAC Tyr GCT Ala	Ala 260 AGA Arg TGG Trp TTT Phe	TTC Phe GTG Val ATC Ile TTC Phe CTC Leu	ACA Thr GCG Ala CTG Leu TCT Ser 310	931 979

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												mcc	אשר	a ሞ b	CAG	1171
ACT	GAG	TGC	TGG	GAC	CTG	AGT	TCG	GGG	AAT	AAG T.ve	LVS	TGG Trp	Ile	Ile	Gln	11.1
Thr	Glu	345		Авр	Leu	ser	350	GIY	VPII	n y n	DŢŪ	355				
GTG	CCC	ATC	CTG	GCA	GCT	ATT	GTG	GTG	AAC	TTT	TTA	CTT	TTT	ATC	AAT	1219
Val	Pro	Ile	Leu	Ala	Ala	Ile	Val	Val	Asn	Phe	Ile	Leu	Phe	Ile	Asn	
	360					365					370					
									000	CNC	ACC.	חממ	CCA	GGG	AGA	1267
ATA	ATC	AGA	GTC	CTG	GCT	ACT	AAA	Len	Ara	Glu	Thr	TAA naA	Ala	Glv	Arg	
375	IIS	Arg	vai	Ten	380	1111	Lyb	200	9	385				-	390	
_																
TGT	GAC	ACG	AGG	CAA	CAG	TAT	AGA	AAG	CTG	CTG	AAG	TCC	ACG	CTA	GTC	1315
Сув	Asp	Thr	Arg			Tyr	Arg	ГÀв	Leu	Leu	ГÀв	Ser	Thr	Leu 40	var	
				39	95				40	00				•	,,	
	3 mg		Cert I	D TTTT	ccc	GTG	CAC	TAC	ATC	GTC	TTC	ATG	GCC	ACG	CCG	1363
CTC	Mot	Pro	Len	Phe	Glv	Val	His	Tyr	Ile	Val	Phe	Met	Ala	Thr	Pro	
Dea	ricc		410	•	,			415					420			
																1411
TAC	ACA	GAA	GTA	TCA	GGG	ATT	CTT	TGG	CAA	GTC	CAA	ATG	CAC	TAT	GAA	1411
Tyr	Thr		Val	Ser	Gly	Ile		Trp	GIn	Val	GIN	Met 435	ure	TAT	GIU .	
		425					430									
ATG	CTC	TTC	AAT	TCA	TTC	CAG	GGA	TTT	TTC	GTT	GCC	ATT	ATA	TAC	TGT	1459
Met	Leu	Phe	Asn	Ser	Phe	Gln	Gly	Phe	Phe	Val	Ala	Ile	Ile	Tyr	Сув	
	440		•			445					450					
						~~~	CCN	CAC	እጥሮ	AAG	D D C	TCA	TGG	AGC	CGA	1507
TTC	TGC	AAT	GGA	GAG	GTA Val	Gin	Ala	Glu	Ile	Lvs	Lvs	Ser	Trp	Ser	Arg	
455		VPII	Gry	GIU	460	·				465	•		_		470	
TGG	ACC	CTG	GCC	TTG	GAC	TTC	AAG	CGG	AAG	GCC	CGG	AGT	GGC	AGC	AGT	1555
Trp	Thr	Leu	Ala			Phe	Lys	Arg	Lys	Ala	Arg	Ser	GIY	ser	Ser 85	
	*			4	75				4	80				-	<b>.</b>	
a CC	TAC	AGC	тат	GGC	CCC	ATG	GTG	TCA	CAT	ACA	AGT	GTC	ACC	AAT	GTG	1603
Thr	Tvr	Ser	Tyr	Gly	Pro	Met	Val	Ser	His	Thr	Ser	Val	Thr	yeu	Val	
			490					495	•				500			
							mmc	TO C	CTC	AGC	CCT	CGA	CTA	GCT	CCT	1651
GGA	CCT	CGA	GGG	GGG	Ten	Ala	Leu	Ser	Leu	Ser	Pro	Arg	Leu	Ala	Pro	
GLY	PIO	505		01,			510					515				
GGG	GCT	GGA	GCC	AGT	GCC	AAT	GGC	CAT	CAC	CAG	TTG	CCT	GGC	TAT	GTG	1699
Gly			Ala	Ser	Ala			His	His	Gln	Leu 530	Pro	GIĀ	Tyr	Val	
	520	)				525	1				336	,				
הממ	ראים:	COT	י קרר	ייים י	יים יי	GAG	AAC	TCA	TTG	CCT	TCA	TCT	GGC	CCA	GAG	1747
I.ve	His	Glv	Ser	Ile	Ser	Glu	Asn	Ser	Leu	Pro	Ser	Ser	Gly	Pro	Glu	
535		1			540					545	,				550	

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				Asp	GAC Asp 55				Asn					Tyr		1795
					CAG Gln		Pro									1843
	GTC Val		TGAG	CCCAT	TAT (	2										1863
(2)					SEQU CHARA				FICA!	rion	NUM	BER:	3	3:		
			(A) I	LENGT	TH:				205	1						
				TYPE:							acio	i				
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	(2	(i) S	EQUE	ENCE	DESC	RIPT	CION	: SE	QUEN	CE II	ON C	3:				
GGC	GGGG	3CC 0	CGG	CGGC	a Go	TCG	AGG	C CG	CGG	CGGC	TGC	CCG	AGG (	SAČGO	CGCCC	60
		rgg (														108
															_	
			Me	et Gl	Ly Al				le A				eu Al	la Le	eu .	
			Me										eu Al		eu .	•
			TGC	et G] 1 CCA	Ly Al	cTC	la Ai	rg I	le Ai 5 GCA	la Pi TAT	co Se GCG	er Le	GTG	la Le 10 GAT	GCG	156
			TGC	et G] 1 CCA	Ly Al	cTC	la Ai	rg I	le Ai 5 GCA	la Pi TAT	co Se GCG	er Le	GTG	la Le 10 GAT	GCG	•
Leu	Leu	Сув 15	TGC Cys	et Gl 1 CCA Pro	GTG Val	CTC Leu	AGC Ser 20	TCC Ser	le Ai 5 GCA Ala	TAT Tyr	CO SE GCG Ala	CTG Leu 25	GTG Val	la Le 10 GAT Asp	GCG Ala	156
Leu GAC	Leu GAT	Cys 15 GTC	TGC Cys TTT	CCA Pro	GTG Val	CTC Leu	AGC Ser 20 GAA	TCC Ser	le Al 5 GCA Ala ATT	TAT Tyr	GCG Ala	CTG Leu 25 CTG	GTG Val	La Le 10 GAT Asp	GCG Ala GCC	•
Leu GAC	Leu GAT	Cys 15 GTC	TGC Cys TTT	CCA Pro	GTG Val	CTC Leu	AGC Ser 20 GAA	TCC Ser	le Al 5 GCA Ala ATT	TAT Tyr	GCG Ala	CTG Leu 25 CTG	GTG Val	La Le 10 GAT Asp	GCG Ala GCC	156
Leu GAC Asp	Leu GAT Asp 30	Cys 15 GTC Val	TGC Cys TTT Phe	CCA Pro	GTG Val AAA Lys	CTC Leu GAG Glu 35	AGC Ser 20 GAA Glu	TCC Ser CAG	GCA Ala ATT	TAT Tyr TTC Phe	GCG Ala CTG Leu	CTG Leu 25 CTG Leu	GTG Val CAC	GAT ASP CGT Arg	GCG Ala GCC Ala	156 204
Leu GAC Asp CAG	GAT Asp 30	Cys 15 GTC Val	TGC Cys TTT Phe	CCA Pro ACC Thr	GTG Val	CTC Leu GAG Glu 35	AGC Ser 20 GAA Glu	TCC Ser CAG Gln	GCA Ala ATT Ile	TAT Tyr TTC Phe	GCG Ala CTG Leu 40	CTG Leu 25 CTG Leu	GTG Val CAC His	GAT Asp CGT Arg	GCC Ala GCC Ala	156
Leu GAC Asp CAG	GAT Asp 30	Cys 15 GTC Val	TGC Cys TTT Phe	CCA Pro ACC Thr	GTG Val AAA Lys	CTC Leu GAG Glu 35	AGC Ser 20 GAA Glu	TCC Ser CAG Gln	GCA Ala ATT Ile	TAT Tyr TTC Phe	GCG Ala CTG Leu 40	CTG Leu 25 CTG Leu	GTG Val CAC His	GAT Asp CGT Arg	GCC Ala GCC Ala	156 204
GAC Asp CAG Gln 45	GAT Asp 30 GCG Ala	Cys 15 GTC Val CAA Gln	TGC Cys TTT Phe TGT Cys	CCA Pro ACC Thr	GTG Val AAA Lys AAG Lys	CTC Leu GAG Glu 35 CTG Leu	AGC Ser 20 GAA Glu CTC Leu	TCC Ser CAG Gln AAG	GCA Ala ATT Ile GAA Glu	TAT Tyr TTC Phe GTT Val	GCG Ala CTG Leu 40 CTG Leu	CTG Leu 25 CTG Leu CAC	GTG Val CAC His	GAT Asp CGT Arg	GCC Ala GCC Ala GCC Ala 60	156 204 252
GAC Asp CAG Gln 45	GAT ABP 30 GCG Ala	Cys 15 GTC Val CAA Gln	TGC Cys TTT Phe TGT Cys	CCA Pro ACC Thr GAC ABP	GTG Val AAA Lys AAG	CTC Leu GAG Glu 35 CTG Leu	AGC Ser 20 GAA Glu CTC Leu	TCC Ser CAG Gln AAG Lys	GCA Ala  ATT Ile  GAA Glu  ACA	TAT Tyr TTC Phe GTT Val 55	GCG Ala CTG Leu 40 CTG Leu GCA	CTG Leu 25 CTG Leu CAC His	GTG Val CAC His ACA Thr	GAT Asp CGT Arg GCA Ala	GCG Ala GCC Ala GCC Ala 60 GGG	156 204
GAC Asp CAG Gln 45	GAT ABP 30 GCG Ala	Cys 15 GTC Val CAA Gln	TGC Cys TTT Phe TGT Cys	CCA Pro ACC Thr GAC ASP	GTG Val AAA Lys AAG Lys 50	CTC Leu GAG Glu 35 CTG Leu	AGC Ser 20 GAA Glu CTC Leu	TCC Ser CAG Gln AAG Lys	GCA Ala  ATT Ile  GAA Glu  ACA Thr	TAT Tyr TTC Phe GTT Val 55	GCG Ala CTG Leu 40 CTG Leu GCA	CTG Leu 25 CTG Leu CAC His	GTG Val CAC His ACA Thr	GAT ASP CGT Arg GCA Ala	GCG Ala GCC Ala GCC Ala 60 GGG	156 204 252
GAC Asp CAG Gln 45 AAC Asn	GAT Asp 30 GCG Ala ATA	Cys 15 GTC Val CAA Gln ATG Met	TGC Cys TTT Phe TGT Cys GAG Glu	CCA Pro ACC Thr GAC Asp	GTG Val AAA Lys AAG Lys 50 GAC Asp	CTC Leu GAG Glu 35 CTG Leu AAG	AGC Ser 20 GAA Glu CTC Leu GGC Gly	TCC Ser CAG Gln AAG Lys	le A. 5 GCA Ala ATT Ile GAA Glu ACA Thr	TAT Tyr TTC Phe GTT Val 55 CCA Pro	GCG Ala CTG Leu 40 CTG Leu .	CTG Leu 25 CTG Leu CAC His	GTG Val CAC His ACA Thr	GAT ASP CGT Arg GCA Ala	GCG Ala GCC Ala GCC Ala 60 GGG Gly	204 252 300
GAC Asp CAG Gln 45 AAC Asn	GAT ABP 30 GCG Ala ATA Ile	Cys 15 GTC Val CAA Gln ATG Met	TGC Cys TTT Phe TGT Cys GAG Glu	CCA Pro ACC Thr GAC ASP	GTG Val AAA Lys AAG Lys 50 GAC Asp	CTC Leu GAG Glu 35 CTG Leu AAG Lys	AGC Ser 20 GAA Glu CTC Leu GGC Gly TCG	TCC Ser CAG Gln AAG Lys TGG Trp GGA	le A. 5 GCA Ala ATT Ile GAA Glu ACA Thr	TAT Tyr TTC Phe GTT Val 55 CCA Pro	GCG Ala  CTG Leu 40  CTG Leu GCA Ala	CTG Leu 25 CTG Leu CAC His	GTG Val CAC His ACA Thr	GAT ASP CGT Arg GCA Ala TCA Ser	GCG Ala GCC Ala GCC Ala 60 GGG Gly 75	156 204 252
GAC Asp CAG Gln 45 AAC Asn	GAT ABP 30 GCG Ala ATA Ile	Cys 15 GTC Val CAA Gln ATG Met	TGC Cys TTT Phe TGT Cys GAG Glu	CCA Pro ACC Thr GAC Asp TCA Ser GAG Glu	GTG Val AAA Lys AAG Lys 50 GAC Asp	CTC Leu GAG Glu 35 CTG Leu AAG Lys	AGC Ser 20 GAA Glu CTC Leu GGC Gly TCG	TCC Ser CAG Gln AAG Lys TGG Trp GGA	le A. 5 GCA Ala ATT Ile GAA Glu ACA Thr	TAT Tyr TTC Phe GTT Val 55 CCA Pro	GCG Ala  CTG Leu 40  CTG Leu GCA Ala	CTG Leu 25 CTG Leu CAC His	GTG Val CAC His ACA Thr	GAT ASP CGT Arg GCA Ala TCA Ser TCT Ser	GCG Ala GCC Ala GCC Ala 60 GGG Gly 75	156 204 252 300
GAC Asp CAG Gln 45 AAC Asn AAG Lys	GAT Asp 30 GCG Ala ATA Ile	Cys 15 GTC Val CAA Gln ATG Met	TGC Cys TTT Phe TGT Cys GAG Glu AAA Lys	CCA Pro ACC Thr GAC Asp	GTG Val AAA Lys AAG Lys 50 GAC Asp	CTC Leu GAG Glu 35 CTG Leu AAG Lys	AGC Ser 20 GAA Glu CTC Leu GGC Gly TCG Ser	TCC Ser CAG Gln AAG Lys TGG Trp GGA Gly 8:	GCA Ala ATT Ile GAA Glu ACA Thr	TAT Tyr TTC Phe GTT Val 55 CCA Pro 70	GCG Ala CTG Leu 40 CTG Leu GCA Ala	CTG Leu 25 CTG Leu CAC His	GTG Val CAC His ACA Thr ACG Thr	GAT ASP CGT Arg GCA Ala TCA Ser	GCG Ala GCC Ala 60 GGG Gly 75 AAA Lys	156 204 252 300
GAC Asp CAG Gln 45 AAC Asn AAG Lys	GAT Asp 30 GCG Ala ATA Ile CCC Pro	Cys 15 GTC Val CAA Gln ATG Met	TGC Cys TTT Phe TGT Cys GAG Glu AAA Lys 80	CCA Pro ACC Thr GAC Asp TCA Ser GAG Glu	GTG Val AAA Lys AAG Lys 50 GAC Asp 55	CTC Leu  GAG Glu 35 CTG Leu  AAG Lys GCA Ala	AGC Ser 20 GAA Glu CTC Leu GGC Gly TCG Ser	TCC Ser CAG Gln AAG Lys TGG Trp GGA Gly 8:	GCA Ala ATT Ile GAA Glu ACA Thr AAG Lys	TAT Tyr TTC Phe GTT Val 55 CCA Pro 70	GCG Ala CTG Leu 40 CTG Leu GCA Ala TAC Tyr	CTG Leu 25 CTG Leu CAC His TCT Ser	GTG Val CAC His ACA Thr ACG Glu 90	GAT ASP CGT Arg GCA Ala TCA Ser Ser	GCG Ala GCC Ala GCC Ala 60 GGG Gly 75 AAA Lys	204 252 300 348

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GTG Val	GAG Glu	GGG	Leu	Tyr	TTG Leu 05	His	Ser	Leu	Ile	Phe 10	ATG Met	Ala	Phe	Phe	Ser 15	
Val 285	Thr	Phe	Phe	Leu	Tyr 290	Phe	Leu	Ala	Thr	Asn 295	Tyr	Tyr	Trp	Ile	Leu 300	1020
GTG	270 ACC	TTC	TTC	CTC	TAC	275 TTC	CTG	GCT	ACC	AAC	280 TAC	TAC	TGG	ATT	CTG	972
CCT Pro	Pro	CCG Pro	GCC Ala	GCT Ala	GCC Ala	Ala	GTA Val	GGC Gly	TAC Tyr	GCT Ala	GGC	TGC Cys	CGC Arg	GTG Val	GCG Ala	924
Ala	Glu	Arg 255	Leu	Thr	Glu	Glu	Glu 260	Leu	Нів	Ile	Ile	Ala 265	Gln	Val	Pro	
•			240					245			ATC		250			876
ATC	TTC	GTG Val	AAG	GAC	GCT	GTG Val	CTC Lev	TAC Tvr	TCT Ser	GGC Glv	TTC Phe	ACG Thr	CTG Leu	GAT Asp	GAG Glu	828
TÄE	116	****	****		25					30		- 9			35	
TAC	ATC	CAC	ATG Met	CAC	ATG Met	TTC Phe	CTG Leu	TCG Ser	TTT Phe	ATG Met	CTG Leu	CGC Arg	GCC Ala	GCG Ala	AGC Ser	780
Ala 205	Val	Leu	Ile	Leu	Ala 210	Tyr	Phe	Arg	Arg	Leu 215	His	Cys	Thr	Arg	Asn 220	
GCT	GTG	CTC	ATC	CTG	GCC	TAT	TTT	AGG	CGG	CTG	CAC	TGC	ACG	ccc	AAC	732
ATG Met	ATC Ile 190	TAC Tyr	ACC Thr	GTG Val	GGA Gly	TAC Tyr 195	Ser	ATG Met	Ser	Leu	GCC Ala 200	Ser	Leu	Thr	Val	364
		175				<b>53.</b> C	180	» mc	m csm	CIDC	CCC	185	CTC	A C C	GTG	684
TTC Phe	ATG Met	ACC Thr	AAT Asn	GAG Glu	ACG Thr	CGG Arg	GAA Glu	CGG Arg	GAG Glu	GTA Val	TTT Phe	Asp	CGC Arg	CTA Leu	GGC Gly	636
vaı	Pro	GIĀ	160	ABN	wrd	THE	ırp	165	noii	*1"			170		-3-	
GTT	CCA	GGG	CAC	AAC	CGG	ACG	TGG	GCC	AAC	TAC	AGC Ser	GAG	TGC	CTC	AAG Lva	588
Lys	Gly	His	Ala	Tyr 14		Arg	Сув	Asp		ABN 50	Gly	ser	IID	15	55	
AAA	GGC	CAT	GCC	TAC	AGA	CGC	TGT	GAC	CGC	AAT	GGC	AGC	TGG	GAG	GTG	540
Glu	Val 125	Val	Ala	Val	Pro 130	Сув	Pro	Asp	Tyr	Ile 135	Tyr	Asp	Phe	Asn	His 140	
CAA	110 GTG	CTC	CCA	СТА	CCT	115 TGT	ccc	САТ	TAC	ATT	120 TAT	GAC	TTC	AAT	CAC	492
CTG Leu	Pro	GAG Glu	TGG Trp	GAC Asp	AAC Asn	Ile	GTT Val	TGC Cyb	TGG Trp	CCA Pro	TTA Leu	GGG Gly	GCA Ala	CCA Pro	GGT Gly	444
														<b>663</b>	000	444

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														CTA Leu		1068
														GCC Ala		1116
														ATC Ile		1164
	Pro				Ser					Phe				ATC Ile		1212
														GGC Gly 395		1260
														TTG Leu		1308
														TTG Leu		1356
TAC Tyr 43	Thr	GAG Glu	GTC Val	TCA Ser	GGG Gly 43	Thr	TTG Leu	TGG Trp	CAG Gln	ATC Ile 44	Gln	ATG Met	CAT His	TAT Tyr	GAG Glu 445	1404
				Ser					Phe					TAC Tyr		1452
TTC Phe	TGC Cys	AAT Asn	GGT Gly 465	Glu	GTG Val	CAG Gln	GCA Ala	GAG Glu 470	ATT	AGG Arg	AAG Lys	TCA Ser	TGG Trp 475	AGC Ser	CGC Arg	1500
			Ala										Gly	AGT Ser		1548
AGC Ser	TAC Tyr 495	Ser	TAT Tyr	GGC Gly	CCA Pro	ATG Met 500	Val	TCT Ser	CAC His	ACG Thr	AGT Ser 505	Val	ACC Thr	AAT Asn	GTG Val	1596
GGC Gly 510	Pro	CGT Arg	GCA Ala	GGA Gly	CTC Leu 515	Ser	CTC Leu	CCC Pro	CTC Leu	AGC Ser 520	Pro	CGC Arg	CTG Leu	CCT Pro	CCT Pro 525	1644

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GCC Ala	ACT Thr	ACC Thr	AAT ABN	GGC Gly 53	His	TCC Ser	CAG Gln	CTG Leu	CCT Pro 53	GIÀ	CAT His	GCC Ala	AAG Lys	CCA Pro 54	Gly	1692
GCT Ala	CCA Pro	GCC Ala	ACT Thr 54	GAG Glu 15	ACT Thr	GAA Glu	Thr	CTA Leu 55	Pro	GTC Val	ACT Thr	ATG Met	WIG	GTT Val		1740
AAG Lys	GAC Asp	GAT Asp 560	GGA Gly	TTC Phe	CTT Leu	AAC Asn	GGC Gly 565	TCC Ser	TGC Cys	TCA Ser	GGC Gly	CTG Leu 570	GAT Asp	GAG Glu	GAG Glu	1788
GCC Ala	TCC Ser 575	GGG Gly	TCT Ser	GCG Ala	CGG Arg	CCG Pro 580	CCT Pro	CCA Pro	TTG Leu	TTG Leu	CAG Gln 585	GAA Glu	GGA Gly	TGG Trp	GAA Glu	1836
	GTC Val 590		TGA	CTGG	GCA (	CTAG	GGGG(	CT A	GACT(	GCTG(	G CC	rggg	CACA			1885
AAG	ממדים	CAA	AAGG	aaga aaaa cctc	TG G	AAGT	GGAC	G AA	GCAG	AGAA	GAA	GGMM	GAT GAG	CTGG GTTT	ACCAGG TGCAGG	1945 2005 2051

What is claimed is:

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### Claims

- Isolated DNA comprising a DNA sequence
- 2 encoding a cell receptor of a vertebrate animal, said
- 3 receptor having an amino acid sequence with at least 30%
- 4 identity to the amino acid sequence shown in FIG. 3.
- 1 2. The isolated DNA of claim 1, wherein said
- 2. DNA sequence encodes substantially all of the amino acid
- 3 sequence shown in FIG. 1 (SEQ. ID NO. 1).
- The isolated DNA of claim 1, wherein said
- 2 DNA sequence encodes substantially all of the amino acid
- 3 sequence shown in FIG. 3 (SEQ. ID NO. 3).
- 1 4. The isolated DNA of claim 1, said isolated
- 2 DNA being (8A6), deposited with the ATCC and designated
- 3 ATCC Accession No. 68570.
- 1 5. The isolated DNA of claim 1, wherein said
- 2 DNA sequence encodes substantially all of the amino acid
- 3 sequence shown in Fig. 6 (SEQ. ID. NO. 4).
- 1 6. The isolated DNA of claim 1, wherein said
- 2 DNA sequence hybridizes to the DNA sequence shown in Fig.
- 3 1 (SEQ. ID NO. 1).
- The isolated DNA of claim 1, wherein said
- 2 DNA sequence hybridizes to the DNA sequence shown in Fig.
- 3 3 (SEQ. ID NO. 3).
- 1 8. The isolated DNA of claim 1, wherein said
- 2 DNA sequence hybridizes to the DNA sequence shown in Fig.
- 3 6 (SEQ. ID NO. 4).

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- 9. A purified preparation of a vector, said
   vector comprising a DNA sequence encoding a parathyroid
- 3 hormone receptor.
- 1 10. A cell containing the isolated DNA of claim
- 2 1.
- 1 11. The cell of claim 10, wherein said cell is
- 2 capable of expressing said cell receptor from said
- 3 isolated DNA.
- 1 12. An essentially homogenous population of
- 2 cells, each of which comprises the isolated DNA of claim
- 3 1.
- 1 13. Isolated DNA comprising a DNA sequence
- 2 encoding a polypeptide capable of binding parathyroid
- 3 hormone or parathyroid-hormone-related protein.
- 1 14. A method for producing a polypeptide, said
- 2 method comprising:
- 3 providing a cell comprising isolated DNA
- 4 encoding a parathyroid hormone receptor or a fragment
- 5 thereof; and
- 6 culturing said cell under conditions
- 7 permitting expression of a polypeptide from said DNA.
- 1 15. A single-stranded DNA comprising a portion
- 2 of a parathyroid hormone receptor gene, said portion
- 3 being at least 18 nucleotides long.
- 1 16. The single-stranded DNA of claim 15, wherein
- 2 said portion is less than all of said parathyroid hormone
- 3 receptor gene.

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- 1 17. The single-stranded DNA of claim 15, wherein
- 2 said DNA is detectably labeled.
- 1 18. A single-stranded DNA comprising a portion
- 2 of a parathyroid hormone receptor cDNA, said portion
- 3 being at least 18 nucleotides long.
- 1 19. The single-stranded DNA of claim 18, wherein
- 2 said DNA is antisense.
- 1 20. Parathyroid hormone receptor produced by
- 2 expression of a recombinant DNA molecule encoding a
- 3 parathyroid hormone receptor.
- 1 21. An essentially purified preparation of the
- 2 parathyroid hormone receptor of claim 20.
- 1 . 22. An essentially purified preparation of the
- 2 parathyroid receptor produced by the expression of the
- 3 DNA of claim 5.
- 1 23. A polypeptide comprising at least six amino
- 2 acids and less than the complete amino acid sequence of a
- 3 parathyroid hormone receptor, said polypeptide capable of
- 4 binding parathyroid hormone or parathyroid hormone-
- 5 related protein.
- 1 24. The polypeptide of claim 23, wherein said
- 2 parathyroid hormone receptor is a human parathyroid
- 3 receptor.
- 1 25. The polypeptide of claim 23, wherein said
- 2 fragment comprises
- 3 (a) TNETREREVFDRLGMIYTVG,
- 4 (b) YLYSGFTLDEAERLTEEEL,

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- 5 (c) VTFFLYFLATNYYWILVEG,
- 6 (d) Y-RATLANTGCWDLSSGHKKWIIQVP,
- 7 (e) PYTEYSGTLWQIQMHYEM,
- 8 (f) DDVFTKEEQIFLLHRAQA,
- 9 (g) FFRLHCTRNY,
- 10 (h) EKKYLWGFTL,
- 11 (i) VLATKLRETNAGRCDTRQQYRKLLK, or
- 12 (j) a fragment of (a) (i) which is capable of
- 13 binding parathyroid hormone or parathyroid hormone-
- 14 related protein.
- 1 26. A therapeutic composition comprising, in a
- 2 pharmaceutically-acceptable carrier, (a) a parathyroid
- 3 hormone receptor or (b) a polypeptide comprising a
- 4 fragment of said receptor.
- 1 27. An antibody capable of forming an immune
- 2 complex with a parathyroid hormone receptor.
- 1 28. A therapeutic composition comprising the
- 2 antibody of claim 27 and a pharmaceutically-acceptable
- 3 carrier.
- 1 29. A method of reducing the level of calcium in
- 2 the blood of a mammal, which method comprises
- 3 administering the therapeutic composition of claim 26 to
- 4 said mammal in a dosage effective to inhibit activation
- 5 by parathyroid hormone or parathyroid hormone-related
- 6 protein of a parathyroid hormone receptor of said mammal.
- 1 30. A method of reducing the level of calcium in
- 2 the blood of a mammal, which method comprises
- 3 administering the therapeutic composition of claim 28 to
- 4 said mammal in a dosage effective to inhibit activation

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by parathyroid hormone or parathyroid hormone-related
protein of a parathyroid hormone receptor of said mammal.

- 31. A method for identifying a compound capable of competing with a parathyroid hormone for binding to a parathyroid hormone receptor, said method comprising:
- 4 (a) contacting the polypeptide of claim 23 with 5 a parathyroid hormone, (i) in the presence or (ii) in the 6 absence of a candidate compound; and
- comparing (i) the level of binding of said 7 polypeptide to said parathyroid hormone in the presence 8 of said candidate compound, with (ii) the level of 9 binding of said polypeptide to said parathyroid hormone 10 in the absence of said candidate compound; a lower level 11 of binding in the presence of said candidate compound 12 than in its absence indicating that said candidate 13 compound is capable of competing with said parathyroid 14 hormone for binding to said receptor. 15
  - 32. A method for identifying a compound capable of competing with a parathyroid hormone-related protein for binding to a parathyroid hormone receptor, said method comprising:
  - (a) contacting the polypeptide of claim 23 with a parathyroid hormone-related protein, (i) in the presence or (ii) in the absence of a candidate compound; and

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(b) comparing (i) the level of binding of said polypeptide to said parathyroid hormone-related protein in the presence of said candidate compound, with (ii) the level of binding of said polypeptide to said parathyroid hormone-related protein in the absence of said candidate compound; a lower level of binding in the presence of said candidate compound than in its absence indicating that said candidate compound is capable of competing with

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said parathyroid hormone-related protein for binding to said receptor.

- 1 33. A method for identifying a compound capable 2 of competing with a parathyroid hormone for binding to a 3 parathyroid hormone receptor, said method comprising:
- parathyroid hormone receptor, said method comprising:

  (a) combining a parathyroid hormone with the

  cell of claim 11, (i) in the presence or (ii) in the

  absence of a candidate compound; and
- 7 (b) comparing (i) the level of binding of said 8 receptor to said parathyroid hormone in the presence of 9 said candidate compound, with (ii) the level of binding
- 10 of said receptor to said parathyroid hormone in the
- 11 absence of said candidate compound; a lower level of
- 12 binding in the presence of said candidate compound than
- 13 in its absence indicating that said candidate compound is
- 14 capable of competing with said parathyroid hormone for
- 15 binding to said receptor.
- 1 34. A compound capable of inhibiting the binding 2 of parathyroid hormone or parathyroid hormone-related 3 protein to a parathyroid receptor on the surface of a 4 cell.
- 1 35. A therapeutic composition comprising the compound of claim 34 and a pharmaceutically-acceptable carrier.
- 1 36. A method for identifying a DNA sequence
  2 homologous to a parathyroid hormone receptor-encoding DNA
  3 sequence, said method comprising:
  4 providing a genomic or cDNA library;
  5 contacting said library with the single-
- contacting said library with the single-6 stranded DNA of claim 18, under conditions permitting

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- 7 hybridization between said single-stranded DNA and a
- 8 homologous DNA sequence in said library; and
- 9 identifying a clone from said library which
- 10 hybridizes to said single-stranded DNA, said
- 11 hybridization being indicative of the presence in said
- 12 clone of a DNA sequence homologous to a parathyroid
- 13 hormone receptor-encoding DNA sequence.
- 1 37. A transgenic non-human vertebrate animal
- 2 bearing a transgene comprising a DNA sequence encoding
- 3 parathyroid hormone receptor or a fragment thereof.
- 38. A diagnostic method comprising:
- 2 (a) obtaining a first blood sample from an
- 3 animal; (b) administering the composition of claim
- 4 35 to said animal;
- 5 (c) obtaining a second blood sample from said
- 6 animal subsequent to said administration of said
- 7 composition; and
- 8 (d) comparing the calcium level in said first
- 9 blood sample with that in said second blood sample, a
- 10 lower calcium level in said second blood sample being
- 11 diagnostic for a parathyroid hormone-related condition.
- 12 39. The isolated DNA of claim 1, wherein said
- 13 DNA sequence encodes a parathyroid hormone receptor.

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- 2 40. The parathyroid hormone receptor of claim 20
- 3 for use in therapy or diagnosis.
- 4 41. The polypeptide of claim 23 for use in
- 5 therapy or diagnosis.
- 6 42. The antibody of claim 27 for use in therapy
- 7 or diagnosis.

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- 8 43. The therapeutic composition of claim 26 for 9 use in therapy for the inhibition of activation by
- 10 parathyroid hormone or parathyroid hormone-related
- 11 protein of a parathyroid hormone receptor of a mammal or
- 12 for the reduction of the level of calcium in the blood of
- 13 a mammal.
- 14 44. The therapeutic composition of claim 28 for
- 15 use in therapy for the inhibition of activation by
- 16 parathyroid hormone or parathyroid hormone-related
- 17 protein of a parathyroid hormone receptor of a mammal or
- 18 for the reduction of the level of calcium in the blood of
- 19 a mammal.
- 20 45. The parathyroid hormone receptor of claim 20
- 21 for use in the manufacture of a medicament for use in
- 22 therapy for the inhibition of activation by parathyroid
- 23 hormone or parathyroid hormone-related protein of a
- 24 parathyroid hormone receptor of a mammal or for the
- 25 reduction of the level of calcium in the blood of a
- 26 mammal.
- 27 46. The polypeptide of claim 23 for use in the
- 28 manufacture of a medicament for use in therapy for the
- 29 inhibition of activation by parathyroid hormone or
- 30 parathyroid hormone-related protein of a parathyroid
- 31 hormone receptor of a mammal or for the reduction of the
- 32 level of calcium in the blood of a mammal.
- 33 47. The antibody of claim 27 for use in the
- 34 manufacture of a medicament for use in therapy for the
- 35 inhibition of activation by parathyroid hormone or
- 36 parathyroid hormone-related protein of a parathyroid
- 37 hormone receptor of a mammal or for the reduction of the
- 38 level of calcium in the blood of a mammal.

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48. A method for identifying a hypercalcemic condition in a patient which is mediated by parathyroid hormone or parathyroid hormone-related protein, the method comprising

- 43 (a) determining the calcium level of a first 44 blood sample from the patient,
- 45 (b) determining the calcium level of a second 46 blood sample from the patient taken at a time subsequent 47 after administration of the therapeutic composition of 48 claim 26, and
- 49 (c) comparing the calcium levels of the two
  50 blood samples, a lower calcium level in the second blood
  51 sample being indicative of a condition related to
  52 parathyroid hormone or parathyroid hormone-related
  53 protein in the patient.
- 49. A method for identifying a hypercalcemic condition in a patient which is mediated by parathyroid hormone or parathyroid hormone-related protein, the method comprising
- 58 (a) determining the calcium level of a first 59 blood sample from the patient,
- 60 (b) determining the calcium level of a second 61 blood sample from the patient taken at a subsequent time 62 after administration of the therapeutic composition of 63 claim 28, and
- 64 (c) comparing the calcium levels of the two
  65 blood samples, a lower calcium level in the second blood
  66 sample being indicative of a condition related to
  67 parathyroid hormone of parathyroid hormone-related
  68 protein in the patient.

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# FIG. i

•	TGGG	CACA	GC C	ACCC	rgtt	G GT	AGTC	CAGG	GGC	CAGC	CCA	CTGA	GCTG	GC A	TATC	AGCTG	śΰ
•	GTGG	cccc	GT T	GGAC	TCGG	כ ככי	TAGG	Ġaac	GGC	GCG	ATG Met		GCG Ala	CCC	CGG Arg	ATC Ile	115
	TCG Ser	CAC His	AGC Ser	CTT Leu 10	GCC '	TTG Leu	CTC Leu	CTC Leu	TGC ' Cys 15	TGC Cys	TCC Ser	GTG Val	CTC Leu	AGC Ser 20	TCC Ser	GTC Val	157
	Tyr	Ala	CTG Leu 25	Val	Хsр	Ala	Asp	QEA 30	741	116	1111	دړد	35	<b>G1u</b>	01		2:-
	Ile	Leu 40	CTG Leu	Arg	Asn	Ala	45	Ald	GIN	Cys	GIU	50	AL 9	Dea	2,0		259
	<b>Val</b> 5 <b>5</b>	Leu	AGG Arg	Val	Pro	61u	Leu	Ala	Giu	Ser	65	בעם	vəħ		1100	70	307
	Arg	Ser	GCA Ala	Lys	Thr 75	Lys	Lys	GIU	Lys	80	YIG	916	בועם	200	85		355
	CAG Gln	GCA Ala	GAG Glu	GAG Glu 90	TCC Ser	AGG Arg	GAA Glu	GTT Val	TCT Ser 35	G <b>AC</b> Asp	AGG Arg	AGC Ser	CGG Arg	CTG Leu 10	<b></b>	GAT Asp	403
	3GC 31y	TTC Phe	TGC Cys 105	Lau	cor Pro	GAG Glu	TGG	ASP 113	AAC AST	ATT	STG Val	TGC Cys	TGG Trp 115	CCT Pro	GCT Ala	GGA Gly	<b>‡</b> :.
	GTG Val	CCC Pro 120	GGC Gly	AAG Lys	GTG Val	GTG Val	GCC Ala 125	val	222 2 <b>7</b> 0	TGC Cys	CCC	GAC Asp 130	TAC	TTC Phe	TAC	GAC Asp	499
	TTC Phe 135	Asn	CAC His	AAA Lys	GGC	CGA Arg 140	Ala	TAT	cgg Arg	CGC Arg	TGT Cys 145	Yab	AGC Ser	AAT Asn	GGC Gly	AGC Ser 150	547
	TGG Trp	GAG Glu	CTG Leu	GTG Val	CCT Pro	Gly	AAC Asn	AAC AST	ccc Arg	ACA Thr 160		GCG Ala	AAT Asn	TAC	AGC Ser 165	GAA Glu	595
	TGT Cys	GTC Val	: AAG Lys	TT1 Phe 170	Leu	ACC Thr	: AAC : Ast	GAC	ACC Thr 175	, ALY	GAA Glu	. CGG . Arg	GAA Glu	GTC Val 180	TTT Phe	GAT Asp	643

## FIG. :

CGC Arg	CTC Leu	GGA Gly 185	ATG Met	ATC Ile	TAC Tyr	ACT Thr	GTG Val 190	GGC	TAC Tyr	TCC Ser	ATC	TCI Ser 195	Let	GGC Gly	TCC Ser	691
CTC Leu	ACT Thr 200	GTG Val	GCT Ala	GTG Val	CTG Leu	ATT Ile 205	CTG Leu	GGT Gly	TAC	TTT	AGG Arg 210	Arg	Leu	CAT His	TGC	739
ACC Thr 215	CGA Arg	AAC Asn	TAC Tyr	ATT Ile	CAC His 220	ATG Met	CAT His	crc Leu	TTC Phe	GTG Val 225	TCC Ser	TTT	ATG Met	CTC Leu	CGG Arg 230	787
GCT Ala	GTA Val	AGC Ser	λTC Ile	TTC Phe 235	λTC Ile	AAG Lys	GAT Asp	3CT λla	GTG Val 240	CTC Leu	TAC Tyr	TCG Ser	GGG Gly	GTT Val 245	TCC Ser	835
ACA Thr	GAT Asp	GAA Glu	ATC Ile 250	GAG Glu	CGC Arg	ATC Ile	ACC	GAG Glu 235	GAG Glu	GAG Glu	CTG Leu	AGG Arg	GCC Ala 260	Phe	ACA Thr	883
Glu	CCT Pro	Pro 265	Pro	Ala	ąsp	Lys	λla 270	Gly	Phe	Val	Gly	Cys 275	Arg	Val	Ala	931
GTA Val	ACC Thr 280	GTC Val	TTC Phe	CTT Leu	TAC Tyr	TTC Phe 285	CTS Leu	ACC	ACC Thr	AAC Asn	TAC Tyr 290	TAC Tyr	TGG Trp	ATC 	CTG Leu	979
Val 295	GAA Glu	Gly	Leu	Tyr	Leu 300	His	Ser	Lau	Ile	Phe 305	Met	Ala	Phe	Phe	Ser 310	1027
GAG Glu	AAA Lys	AAG Lys	TAT Tyr	CTC Leu 315	TGG Trp	GGT Gly	TTC Pha	ACA The	TTA Leu 320	TTT Phe	GGC Gly	TGG Trp	GC	CTC Leu 325	CCT Pro	1075
Ala	ars Fal	Phe	7a1 330	Ala	Val	7rp	a. <u>.</u>	:25	"al	Arg	Ala	Thr	Leu 340	GCC Ala	Asn	1123
Thr	GAG Glu	Cys 345	dır	Asp	Leu	Ser	Ser 350	317	Asn	Lys	Lys	Trp 355	Ile	Ile	Gln	1171
Val	CCC Pro 360	Ile	Leu	Ala	Ala	11e 365	Val	∵al	Asn _.	Phe	Ile 370	Leu	Phe	Ile	Asn	1219
A <b>TA</b> Ile 375	ATC Ile	AGA Arg	GTC Val	CTG Leu	GCT Ala 380	ACT Thr	AAA Lys	CTC Leu	CG <b>G</b> A <b>r</b> g	GAG Glu 38 <b>5</b>	ACC Thr	AAT Asn	GCA Ala	G <b>GG</b> Gly	AGA Arg 390	1267

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TGT Cys	GAC Asp	ACG Thr	AGG Arg	CAA Gln 395	CAG Gln	TAT Tyr	AGA Arg	AAG Lys	CTG Leu 400	CTG Leu	AAG Lys	TCC Ser	ACG Thr	CTA Leu 405	GTC Val	1315
CTC Leu	ATG Met	CCG Pro	CTA Leu 410	TTT Phe	GGG Gly	GTG Val	CAC His	TAC Tyr 415	ATC Ile	GTC Val	TTC Phe	ATG Met	GCC Ala 420	ACG Thr	CCG Pro	1253
TAC Tyr	ACA Thr	GAA Glu 425	GTA Val	TCA Ser	GGG Gly	ATT Ile	CTT Leu 430	TGG Trp	CAA Gln	GTC Val	CAA Gln	ATG Met 435	CAC His	TAT Tyr	GAA Glu	1411
ATG Met	CTC Leu 440	TTC Phe	AAT Asn	TCA Ser	TTC Phe	CAG Gln 445	GGA Gly	TTT Phe	TTC Phe	GTT Val	GCC Ala 450	ATT Ile	ATA Ile	TAC Tyr	TGT Cys	1459
	TGC Cys															1507
	ACC Thr															1555
ACC Thr																1603
GGA Gly													TAGC	TCCI	GG	1652
GGCT	GGAG	CC A	GTGC	CAAT	G GC	CATO	ACCA	GTI	cct	rgge	TATG	TGAA	GC A	TGGT	TCCAT	1712
TTCT	GAGA	AC I	CATI	cci	T CA	TCTG	GCCC	: AGA	.GCCT	rggc	ACCA	AAGA	TG A	CGGG	TATCT	1770
CAAT	GGCT	cr e	GACI	TTAI	'G AG	CCAA	TGGT	TGG	GGAA	CAG	cccc	crec	ac t	ccie	GAGGA	1832
GGAG	AGAG	AG A	CAGI	CATO	T GA	CCCA	TATO	:								1862

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TGG	GCAC	AGC (	CACCO	TGT	G GI	TAGTO	CAGO	GGG	CCAG	CCA	CTG	AGCT	GGC (	ATAT	CAGCTO	60
GTG	GCCC	CGT 7	rggac	CTCGC	c co	TAGO	GAA(	C GGG	CGGC	Met	G GG E Gly	A GCG Y Ala	s cc	C CG	G ATC g Ile 5	115
TCG Ser	CAC His	AGC Ser	CTT Leu 10	GCC Ala	TTG Leu	CTC Leu	CTC Leu	TGC Cys	TGC Cys	TCC Ser	GTG Val	CTC Leu	AGC Ser 20	TCC Ser	GTC Val	163
TAC Tyr	GCA Ala	CTG Leu 25	GTG Val	GAT Asp	GCC Ala	GAT Asp	JAT Asp 30	GTC Val	λ <b>TA</b> Ile	ACG Thr	AAG Lys	GAG Glu 35	GA <b>G</b> Glu	CAG Gln	ATC Ile	211
ATT Ile	CTT Leu 40	CTG Leu	CGC Arg	AAT Asn	GCC Ala	CAG Gln 45	GCC Ala	CAG Gln	TGT Cys	GAG Glu	CAG Gln 50	CGC Arg	CTG Leu	AAA Lys	GAG Glu	259
GTC Val 55	CTC Leu	AGG Arg	GTC Val	CCT Pro	GAA Glu 60	CTT Leu	GCT Ala	GAA Glu	TCT Ser	GCC Ala 65	AAA Lys	GAC Asp	TGG Trp	ATG Met	TCA Ser 70	307
AGG Arg	TCT Ser	GCA Ala	AAG Lys	ACA Thr 75	AAG Lys	AAG Lys	GAG Glu	lys Lys	CCT Pro 80	GCA Ala	GAA Glu	AAG Lys	CTT Leu	TAT Tyr 85	CCC Pro	355
CAG Gln	GCA Ala	GAG Glu	GAG Glu 90	TCC Ser	AGG Arg	GAA Glu	GTT Val	TCT Ser	GAC Asp	AGG Arg	AGC Ser	cgg Arg	CTG Leu 100	Gln	GAT Asp	403
GGC 317	TTC	TGC Cys 105	CTA Leu	CCT Pro	GAG Glu	TGG	GAC ST	AAC .sm	ATT	GTG Val	TGC Cys	TGG Trp 115	CCT Pro	GCT Ala	GGA Gly	451
GTG Val	CCC Pro 120	GGC Gly	AAG Lys	GTG Val	GTG Val	GCC Ala 125	373	:00	CÀR	CCC Pro	GAC Asp 130	TAC Tyr	TTC Phe	TAC Tyr	GAC Asp	499
TTC Phe 135	AAC Asn	CAC His	AAA Lys	GGC Gly	CGA Arg 140	GCC Ala	TAT Tyr	caa Arg	CGC Arg	TGT Cys 145	GAC Asp	AGC Ser	AAT Asn	GGC Gly	AGC Ser 150	547
TGG Trp	GAG Glu	CTG Leu	GTG Val	CCT Pro 155	GGG Gly	AAC Asn	AAC Asn	csg Arg	ACA Thr 160	TGG Trp	GCG Ala	AAT Asn	TAC Tyr	AGC Ser 165	GAA Glu	595
TGT Cys	GTC Val	AAG Lys	TTT Phe 170	Leu	ACC	AAC Asn	GAG Glu	ACC Thr 175	cgg Arg	GAA Glu	CGG Arg	GAA Glu	GTC Val 180	TTT Phe	GAT Asp	643

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FIG. 2

CGC CTC GGA ATG ATC TAC ACT GTG GGC TAC TCC ATC TCT CTG GGC TCC 591 Arg Leu Gly Met Ile Tyr Thr Val Gly Tyr Ser Ile Ser Leu Gly Ser CTC ACT GTG GCT GTG CTG ATT CTG GGT TAC TTT AGG AGG TTA CAT TGC Leu Thr Val Ala Val Leu Ile Leu Gly Tyr Phe Arg Arg Leu His Cys 205 ACC CGA AAC TAC ATT CAC ATG CAT CTC TTC GTG TCC TTT ATG CTC CGG 737 Thr Arg Asn Tyr Ile His Met His Leu Phe Val Ser Phe Met Leu Arg GCT GTA AGC ATC TTC ATC AAG GAT GCT GTG CTC TAC TCG GGG GTT TCC 335 Ala Val Ser Ile Phe Ile Lys Asp Ala Val Leu Tyr Ser Gly Val Ser 235 ACA GAT GAA ATC GAG CGC ATC ACC GAG GAG GAG CTG AGG GCC TTC ACA 383 Thr Asp Glu Ile Glu Arg Ile Thr Glu Glu Glu Leu Arg Ala Phe Thr 250 260 GAG CCT CCC CCT GCT GAC AAG GCG GGT TTT GTG GGC TGC AGA GTG GCG 931 Glu Pro Pro Pro Ala Asp Lys Ala Gly Phe Val Gly Cys Arg Val Ala 265 270 GTA ACC GTC TTC CTT TAC TTC CTG ACC ACC AAC TAC TAC TGG ATC CTG 979 Val Thr Val Phe Leu Tyr Phe Leu Thr Thr Asn Tyr Tyr Trp Ile Leu 280 285 GTG GAA GGC CTC TAC CTT CAC AGC CTC ATC TTC ATG GCT TTT TTC TCT 1027 Val Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Phe Ser 295 300 GAG AAA AAG TAT CTC TGG GGT TTC ACA TTA TTT GGC TGG GGC CTC CCT 1075 Glu Lys Lys Tyr Leu Trp Gly Phe Thr Leu Phe Gly Trp Gly Leu Pro GCC GTG TTT GTC GCT GTG TGG GTG ACC GTG AGG GCT ACA CTG GCC AAC Ala Val Phe Val Ala Val Trp Val Thr Val Arg Ala Thr Leu Ala Asn 1123 330 ACT GAG TGC TGG GAC CTG AGT TCG GGG AAT AAG AAA TGG ATC ATA CAG 1171 Thr Glu Cys Trp Asp Leu Ser Ser Gly Asn Lys Lys Trp Ile Ile Gln GTG CCC ATC CTG GCA GCT ATT GTG GTG AAC TTT ATT CTT TTT ATC AAT 1219 Val Pro Ile Leu Ala Ala Ile Val Val Asn Phe Ile Leu Phe Ile Asn 365 370 ATA ATC AGA GTC CTG GCT ACT AAA CTC CGG GAG ACC AAT GCA GGG AGA 1267 Ile Ile Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg 380 385

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TGT Cys	GAC Asp	ACG Thr	AGG Arg	CAA Gln 395	CAG Gln	TAT Tyr	AGA Arg	AAG Lys	CTG Leu 400	CTG Leu	AAG Lys	TCC Ser	ACG Thr	CTA Leu 405	GTC Val	1315
CTC Leu	ATG Met	CCG Pro	CTA Leu 410	TTT Phe	GGG Gly	GTG Val	CAC His	TAC Tyr 415	ATC Ila	GTC Val	TTC Phe	ATG Met	GCC Ala 420	ACG Thr	CCG Pro	1363
TAC Tyr	ACA Thr	GAA Glu 425	GTA Val	TCA Ser	GGG Gly	ATT Ile	CTT Leu 430	TTP TGG	CAA Gln	GTC Val	CAA Gln	ATG Met 435	CAC His	TAT Tyr	GAA Glu	1411
ATG Met	CTC Leu 440	TTC Phe	AAT Asn	TCA Ser	TTC Phe	CAG Gln 445	GGA Gly	TTT Phe	TTC Phe	GTT Val	GCC Ala 450	ATT Ile	ATA Ile	TAC Tyr	TGT Cys	1459
Phe 455	Cys	Asn	Gly	Glu	Val 460	Gln	Ala	GAG Glu	Ile	Lys 465	Lys	ser	Trp	ser	470	1507
Trp	Thr	Leu	Ala	Leu 475	Asp	Phe	Lys	CGG Arg	Lys 480	Ala	Arg	Ser	GIÀ	Ser 485	Ser	1555
ACC	TAC Tyr	AGC Ser	TAT Tyr 490	Gly	CCC Pro	ATG Met	GTG Val	TCA Ser 495	His	ACA Thr	AGT Ser	GTC Val	ACC Thr 500	Asn	GTG Val	1603
GGA Gly	CCT Pro	CGA Arg 505	GGG Gly	GGG Gly	CTG Leu	GCC Ala	TTG Leu 510	TCC Ser	CTC Leu	AGC Ser	CCT Pro	CGA Arg 515	CTA Leu	GCT Ala	CCT Pro	1651
GGG Gly	GCT Ala 520	GGA Gly	GCC Ala	AGT Ser	GCC Ala	AAT Asn 525	GGC Gly	CAT %15	CAC	CAG Gln	TTG Leu 530	CCT Pro	GGC	TAT	GTG Val	1699
AAG Lys 535	CAT	SGT Gly	TCC Ser	ATT	TCT Ser 540	Glu	AAC Asn	:ch	TTG Leu	CCT Pro 545	TCA Ser	TCT Ser	GGC Gly	CCA Pro	GAG Glu 550	1747
Pro	Gly	Thr	Lys	Asp 55	Asp 5	Gly	Tyr	c <b>rc</b> Lau	36 56	O GIÀ	Ser	GIĀ	Leu	56!	5	1795
CCA Pro	ATG Met	GTT Val	GGG Gly 57	Glu	CAG Gln	CCC Pro	ccr Pro	CCA Pro 57	Leu	CTG Leu	GAG Glu	GAG Glu	GAG Glu 58	Arg	GA <b>G</b> Glu	1843
		ATG Met 585		.ccca	TAT	С										1863

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1 of 3 FIG. 3

GGC	GGGG	GCC (	SCGG	CGGC	GA GO	TCGC	GAGG	CGC	GCGG	CGGC	TGC	CCCG.	AGG (	GACG	ceeccc	60
TAG	GCGG'	rgg (	CG AT	rg go et gi 1	eg go Ly Al	CC GC la Al	cc co La Ai	3G A1 3g I1	rc Go le Al	CA CO la Pi	CC AG	er L	TG G eu A 10	CG C la L	TC eu	108
CTA Leu	CTC Leu	TGC Cys 15	TGC Cys	CCA Pro	GTG Val	CTC Leu	AGC Ser 20	TCC Ser	GCA Ala	TAT Tyr	GCG Ala	CTG Leu 25	GTG Val	GAT Asp	GCG Ala	133
GAC Asp	GAT Asp 30	GTC Val	TTT Phe	ACC Thr	AAA Lys	GAG Glu 35	GAA Glu	CAG Gln	ATT Ile	TTC Phe	CTG Leu 40	CTG Leu	CAC His	CGT Arg	GCC Ala	204
	GCG Ala															Ţ
	ATA Ile															300
AAG Lys	CCC Pro	AGG Arg	AAA Lys 80	GAG Glu	AAG Lys	GCA Ala	TCG Ser	GGA Gly 85	AAG Lys	TTC Phe	TAC Tyr	CCT Pro	GAG Glu 90	TCT Ser	AAA Lys	348
GAG Glu	AAC Asn	AAG Lys 95	GAC Asp	GTG Val	CCC Pro	ACC Thr	GGC Gly 100	Ser	AGG Arg	CGC Arg	AGA Arg	GGG Gly 105	Arg	CCC Pro	TGT Cys	396
CTG Leu	CCC Pro 110	GAG Glu	TGG Trp	GAC Asp	AAC Asn	ATC Ile 115	GTT Val	TGC Cys	TGG Trp	CCA Pro	TTA Leu 120	GGG Gly	GCA Ala	CCA Pro	GGT Gly	444
GAA 31:1 125	GTG Val	GTG "al	GCA Ala	GTA ∵al	CCT Pro 130	TGT C;s	ccc ?rs	GAT Asp	TAC	ATT Ila IJS	TAT	G <b>AC</b> Asp	TTC Phe	AAT Asn	CAC His 140	491
AAA Lys	GGC Gly	CAT His	GCC Ala	TAC Tyr 145	λGA Arg	CGC Arg	TGT Cys	GAC Asp	CGC Arg 150	λΑΤ Asn	GGC Gly	λGC Ser	TGG Trp	GAG Glu 155	GTG Val	54C
GTT Val	CCA Pro	GGG Gly	CAC His 160	AAC Asn	CGG Arg	ACG Thr	TGG Trp	GCC Ala 165	AAC Asn	TAC Tyr	AGC Ser	GAG Glu	TGC Cys 170	CTC Leu	AAG Lys	588
TTC Phe	ATG Met	ACC Thr 175	AAT Asn	GAG Glu	ACG Thr	cGG	GAA Glu 180	cgg Arg	GAG Glu	GTA Val	Tir	GAC Asp 185	CGC Arg	CTA Leu	GGC Gly	636
ATG Met	ATC Ile 190	TAC TYT	ACC	GTG Val	GGA Gly	TAC TYT 195	TCC Ser	ATG Met	TCT Ser	CTC	GCC Ala 200	TCC Ser	CTC Leu	ACG Thr	GTG Val	684

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2 f 3 FIG. 3

GCT , Ala 205	GTG Val	CTC Leu	ATC Ile	CTG Leu	GCC Ala 210	TAT Tyr	TTT Phe	AGG Arg	CGG Arg	CTG Leu 215	CAC His	TGC Cys	ACG Thr	CGC Arg	AAC Asn 220	732
. TAC Tyr	ATC Ile	CAC His	ATG Met	CAC His 225	ATG Met	TTC Phe	CTG Leu	TCG Ser	TTT Phe 230	ATG Met	CTG Leu	CGC Arg	GCC Ala	GCG Ala 235	AGC Ser	780
ATC Ile	TTC Phe	GTG Val	AAG Lys 240	GAC Asp	GCT Ala	GTG Val	CTC Leu	TAC Tyr 245	TCT Ser	GGC Gly	TTC Phe	ACG Thr	CTG Leu 250	GAT Asp	GAG Glu	828
.GCC Ala	GAG Glu	CGC Arg 255	CTC Leu	ACA Thr	GAG Glu	GAA Glu	GAG Glu 260	TTG Lau	CAC His	ATC Ile	ATC Ile	GCG Ala 265	CAG Gln	GTG Val	CCA Pro	876
ccr Pro	CCG Pro 270	CCG Pro	GCC Ala	GCT Ala	GCC Ala	GCC Ala 275	GTA Val	GGC Gly	TAC Tyr	GCT Ala	GGC Gly 280	TGC Cys	CGC Arg	GTG Val	GCG Ala	924
	ACC Thr															972
GTG Val	GAG Glu	GGG Gly	CTG Leu 305	TAC Tyr	TTG Leu	CAC His	λGC Ser	CTC Leu 310	ATC Ile	TTC Phe	ATG Met	GCC Ala	TTT Phe 315	TTC Phe	TCA Ser	1020
	AAG Lys															1068
GCT Ala	GTC Val 335	TTC Fhe	GTG Val	GCT Ala	GTG Val	TGG Tzp 340	GTC Val	3 <b>3T</b> 317	GTC Wal	λ <b>GA</b> λrg	GCA Ala 345	ACC Thr	TTG Leu	GCC Ala	AAC Asn	1116
ACT Thr 350	GGG Gly	CY'S	TCG TEP	GAT Asp	CTG Leu 355	AGC Ser	TCC Ser	300 317	IAC Ris	AAG Lys 360	AAG Lys	TGG Trp	ATC Ile	ATC Ile	CAG Gln 365	1164
GTG Val	CCC Pro	ATC Ile	CTG Leu	GCA Ala 370	TCT Ser	GTT Val	GTG Val	CTC Leu	AAC Asn 375	TTC Phe	ATC Ile	CTT Leu	TTT Phe	ATC Ile 380	AAC Asn	1212
	ATC Ile															1260
TGT Cys	GAC Asp	ACC Thr 400	AGG Arg	CAG Gln	CAG Gln	TAC Tyr	CGG Arg 405	Γλε γγα	CTG Leu	CTC Leu	AGG Arg	TCC Ser 410	ACG Thr	TTG Leu	GTG Val	1308

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CTC GTG CCG CTC TTT GGT GTC CAC TAC ACC GTC TTC ATG GCC TTG CCG Leu Val Pro Leu Phe Gly Val His Tyr Thr Val Phe Met Ala Leu Pro 415 420 425	1356
TAC ACC GAG GTC TCA GGG ACA TTG TGG CAG ATC CAG ATG CAT TAT GAG Tyr Thr Glu Val Ser Gly Thr Leu Trp Gln Ile Gln Met His Tyr Glu 430 435 440 445	1404
ATG CTC TTC AAC TCC TTC CAG GGA TTT TTT GTT GCC ATC ATA TAC TGT Met Leu Phe Asn Ser Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys 450 455	1452
TTC TGC AAT GGT GAG GTG CAG GCA GAG ATT AGG AAG TCA TGG AGC CGC Phe Cys Asn Gly Glu Val Gln Ala Glu Ile Arg Lys Ser Trp Ser Arg 465 470 175	1500
TGG ACA CTG GCG TTG GAC TTC AAG CGC AAA GCA CGA AGT GGG AGT AGC Trp Thr Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Ser 480 485 490	15+8
AGC TAC AGC TAT GGC CCA ATG GTG TCT CAC ACG AGT GTG ACC AAT GTG Ser Tyr Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val 495 500 505	1596
GGC CCC CGT GCA GGA CTC AGC CTC CCC CTC AGC CCC CGC CTG CCT CCT Gly Pro Arg Ala Gly Leu Ser Leu Pro Leu Ser Pro Arg Leu Pro Pro 510 525	1644
GCC ACT ACC AAT GGC CAC TCC CAG CTG CCT GGC CAT GCC AAG CCA GGG Ala Thr Thr Asn Gly His Ser Gln Leu Pro Gly His Ala Lys Pro Gly 530 535 540	1692
GCT CCA GCC ACT GAG ACT GAA ACC CTA CCA GTC ACT ATG GCG GTT CCC Ala Pro Ala Thr Glu Thr Glu Thr Lau Pro Val Thr Met Ala Val Pro 545	1740
AAG GAC GAT GGA ITC CTT AAC SGC TSC TSC TSL GGC CTG GAT GAG GAG Lys Asp Asp Gly Phe Leu Asn Gly Ser Cys Ser Gly Leu Asp Glu Glu 560 565 570	1783
GCC TCC GGG TCT GCG CGG CCG CCT CCA TTG TTG CAG GAA GGA TGG GAA Ala Ser Gly Ser Ala Arg Pro Pro Pro Leu Leu Gln Glu Gly Trp Glu 575 580 585	1836
ACA GTC ATG TGACTGGGCA CTAGGGGGGCT AGACTGCTGG CCTGGGCACA 1885 Thr Val Met 590	
TGGACAGATG GACCAAGAAG CCAGTGTTTG GCTGGTTGTC TATTCGGGAT CTGGACCAGG	1945
AAGATAACAA AAGGAAAATG GAAGTGGACG AAGCAGAGAA GAAGGAAGAG GTTTTGCAGG	2005
AATTAAATAT GTTTCCTCAG TTGGATGATG AGGACACAAG GAAGGC	2051

Fig. 4

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1 MGAARIAPSLALLLCCPVLSSAYALVDADDVFTREZQIFLLHRAQAQCDX 50
   51 LLKEVLHTAANIHESDKGWTPASTSGKPRKEKASGKFYPESKENKDVPTG 100
  101 SRRRGRPCLFENDNIVCWPLGAPGEVVAVPCPDYIYDFNHKGEAYRRCDR 150
  98 SRLQDGFCLPEWDNIVCWPAGVPGKVVAVPCPDYFYDFNHKGRAYRRCDS 147
 151 MGSWEVVPGHNRTWANYSECLEFHTNETREREVFDRLGHIYTVGYSHSLA 200
    148 NGSWELVPGNARTMANYSECVRPLTHETREREVPDRLGHIYTYGYSISLG 197
 201 SLTVAVLILAYFRRLHCTRHYIHHHHFLSFHLRAASIFVKDAVLYSGFTL 250
 251 DEAERLTEEELHIJAQVPPPPAAAAVGYAGCRVAVTFFLYFLATNYYWIL 300
 301 VEGLYLHSLIFHAFFSEKKYLWGFTIFGWGLFAVFVAVWVGVRATLANTG 350
151 CHDLSSGHKKWIIQVPILASVVLNFILFINIIRVLATKLRETNAGRCDTR 400
401 OOYRKLLASTLVLVPLFGVHYTVFMALPYTEVSGTLWQIQHHYEMLFHSF 450
   195 OQYRKLLXSTLVLHPLFGVHYIVFMATPYTTVSGILWQVQHHYENLFHSF 444
451 CGFFVAIIYCFCMGEVQAEIRKSWSRWTLALDFRRKARSGSSSYSYGPMV 500
445 QGFFVALLYCFCNGEVQAEIKKSWSRWTLALDFKRKARSGSSTYSYGPNV 494
501 SHTSVTNYGFPAGLSLPLSPRLPP...ATTNGHSQLPGHAKPGAPATETE 547
548 TLPVTMAVPRDDGFLNGSCSGLDEEASGEARPPLLQEGWETVN. 591
545 PSSGFEPGTXDDGYLNG..SGLYEPMYG.ECPPLLZEERETYM 586
   Gap Weight: 3.000
Length Weight: 0.100
                       Average Match: 0.540
                     Average Mismacon: -0.396
        Quality: 712.2
Ratio: 1.215
                            Length:
                                    595
                              Saps:
Percent Similarity: 87.113
                     Percent Identity: 77.835
```

Fig. 5

					•	
216	#CAADTADE!	ALLLCCPVLS	SAYALVDADD	VITREEQIFL	LHRAQAQCDK	50
R15 Oko	WELDSTOUCT	ALLICCEVIE	SUYAL.VDADD	VITREEULLE	LKKAUAUCED	50
Okh	MCAPRISHSI.	ALLLCCSVLS	SVYALVDADD	VITKEEQIIL	LRNAQAQCEQ	50
UXII		A				
R15	LLKEVLHTAA	NIMESDEGHT	PASTSGKPRK	EKASGKFYPE	SKENKOVPTG	100
Oko	DIVENTO UP	MURESALIS	MSRSAKTEK	EKPAEKLYPO	AEESREVSDR	97
Okh	RLKEVLR.VP	ELAESAKOW.	. MSRSAKTKK	EXPARKLYPO	ARESREVSOR	97
	•	•		CORVEYDEND	*CDAVDDCDD	160
R15	SARRGRPCLP	EMDNIACMAL	GAPGEVVAVP	CADITIDING	KGHAYRRCDR KGRAYRRCDS	147
Oko	SRLQDGFCLP	EWDNIVCWPA	CALCELLIAND	CONVENDENCE	KGRAYRRCDS	147
Okh	SRLQDGFCLP	EMDNIACMAY	- B		KOIGHTHACDS	,
	N	и .и .	N			
R15	NCCHERRICH	NETHANYSEC	IXENTNETRE	REVFDRLGMI	YTVGYSMSLA	200
Oko	NCCWELUPON	NETWANYSEC	VEFLINETRE	REVPDRLGAI	YTVGYSISLG	197
Okh	NGSWELVPGN	NRTWANYSEC	VEFLTNETRE	REVFDRLGHI	YTVGYSISLG	197
OXII	.403#EL710#		****			
R15	SLTVAVLILA	YFRRLHCTRN	YIHMRMFLSF	HLRAASIFVK	DAVLYSGFTL	250
Oko	CT TITALIT TT C	VPDDIECTEN	YTHMHLFVSF	MLRAVSIFIX	DAVLYSGVST	247
Okh	SLTVAVLILG	YFRRLHCTRN	YIEMHLEVSE	MLRAVSIPIK	DAVLYSGVST	247
	C			D		
					PLATNYYWIL	300
R15			DOINTICTUC	CRVAVTVFLY	FLTTNYYWIL.	<b>294</b>
Oko Okh	DETERITEE	IDARTE D	PPADRAGFVG	CRVAVTVFLY	FLTTNYYWIL	294
OKII	DETERTIES			E		
R15	VEGLYLHSLI	FMAFFSEKKY	LWGFTIFGWG	LPAVPVAVWV	GVRATLANTG	350
Oko		CMACECETTY	CHARTE FORG	LPAVFVAVWV	TVRATLANTE	344
Okh	VEGLYLHSLI	FMAFFSEKKY	LWGPTLIGNG	<b>TAVALAWAMA</b>	TYRATLANTE	344
				G		
	•			******	ETNAGRODTR	400
R15	CWDLSSGHKK	WIIQVPILAS	VVLNFILFIN	TIRVILATRIR	ETNAGRODTR	394
Oko	CWDLSSGNKK	WILGALITY	TUDNETT.FIN	SIRVLATELE.	ETNAGRODER	394
Okh	CMDESSGMKK	MIIGALIEVA	B			
<b>R15</b>	QQYRKLLRST	LULUPLEGUN			RHYEKLINSI	450
Oko			AIALWPLIT	EASCLFMOID		
	AAVETT ! TET	7 177 WOT ECTIO	VIUPHATPYT	SYSGILWOVO	RHYERLFNSF	444
Okh	AAVETT ! TET	LVLMPLFGVH LVLMPLFGVH	YIVFHATPYT	SYSGILWOVO	MEYEMLENSE	444
Okh	AAVETT ! TET	7 177 WOT ECTIO	YIVFHATPYT	SYSGILWOVO	RHYERLFNSF	444
	QQYRKLLXST QQYRKLLXST	LVLMPLFGVH LVLMPLFGVH	YIVFMATPYT	EASCITMOAO	ABYEMLENSE 	444
R15	QQYRKLLXST QQYRKLLXST	LVLMPLFGVH LVLMPLFGVH	YIVFMATPYT YIVFMATPYT	EVSGILWQVQ	ABYEALFNSF ABYEALFNSF 	444
R15 Oko	QQYRKLLKST QQYRKLLKST QGFFVAIIYC	LVLMPLFGVH LVLMPLFGVH 	YIVFMATPYT YIVFMATPYT RKSWSRWTLA	EVSGILWQVQ EVSGILWQVQ LDFKRKARSG LDFKRKARSG	SSSYSYGPHV SSTYSYGPHV	500 494
R15	QQYRKLLKST QQYRKLLKST QGFFVAIIYC QGFFVAIIYC QGFFVAIIYC	LVLMPLFGVH LVLMPLFGVH 	YIVFMATPYT YIVFMATPYT RKSWSRWTLA	EVSGILWQVQ EVSGILWQVQ LDFKRKARSG LDFKRKARSG	ABYEALFNSF ABYEALFNSF 	500 494
R15 Oko	QQYRKLLKST QQYRKLLKST QGFFVAIIYC QGFFVAIIYC QGFFVAIIYC	LVLMPLFGVH LVLMPLFGVH 	YIVFHATPYT YIVFHATPYT RKSWSRWTLA KKSWSRWTLA KKSWSRWTLA	EVSGILWOVQ EVSGILWOVQ LDFKRKARSG LDFKRKARSG LDFKRKARSG	SSSYSYGPHV SSTYSYGPHV SSTYSYGPHV	500 494 494
R15 Oko Okh	QQYRKLLXST QQYRKLLXST QGFFVAIIYC QGFFVAIIYC QGFFVAIIYC	LVLMPLFGVH LVLMPLFGVH FCNGEVQAEI FCNGEVQAEI FCNGEVQAEI	YIVFMATPYT YIVFMATPYT RKSWSRWTLA KKSWSRWTLA KKSWSRWTLA	EVSGILWOVO EVSGILWOVO LDFKRKARSG LDFKRKARSG LDFKRKARSG	ABYENLENSE MEYENLENSE SSSYSYGPHV SSTYSYGPHV SSTYSYGPHV KPGAPATETE	500 494 494 547
R15 Oko Okh	QQYRKLLXST QQYRKLLXST QGFFVAIIYC QGFFVAIIYC GGFFVAIIYC J SHTSVTNVGP	LVLMPLFGVH LVLMPLFGVH FCNGEVQAEI FCNGEVQAEI FCNGEVQAEI RAGLSLPLSP	YIVFMATPYT YIVFMATPYT RKSWSRWTLA KKSWSRWTLA KKSWSRWTLA RLPPATT RLAPGAGASA	EVSGILWOVO EVSGILWOVO LDFKRKARSG LDFKRKARSG LDFKRKARSG NGBSQLPGBA NGBSQLPGBA	ABYENLENSE MEYENLENSE SSSYSYGPHV SSTYSYGPHV SSTYSYGPHV KPGAPATETE	500 494 494 547 547
R15 Oko Okh R15 Oko	QQYRKLLXST QQYRKLLXST QGFFVAIIYC QGFFVAIIYC GGFFVAIIYC J SHTSVTNVGP	LVLMPLFGVH LVLMPLFGVH FCNGEVQAEI FCNGEVQAEI FCNGEVQAEI RAGLSLPLSP	YIVFMATPYT YIVFMATPYT RKSWSRWTLA KKSWSRWTLA KKSWSRWTLA RLPPATT RLAPGAGASA	EVSGILWOVO EVSGILWOVO LDFKRKARSG LDFKRKARSG LDFKRKARSG NGBSQLPGBA NGBSQLPGBA	SSSYSYGPHV SSTYSYGPHV SSTYSYGPHV	500 494 494 547
R15 Oko Okh	QQYRKLLXST QQYRKLLXST QGFFVAIIYC QGFFVAIIYC GGFFVAIIYC J SHTSVTNVGP	LVLMPLFGVH LVLMPLFGVH FCNGEVQAEI FCNGEVQAEI FCNGEVQAEI	YIVFMATPYT YIVFMATPYT RKSWSRWTLA KKSWSRWTLA KKSWSRWTLA RLPPATT RLAPGAGASA	EVSGILWOVO EVSGILWOVO LDFKRKARSG LDFKRKARSG LDFKRKARSG NGBSQLPGBA NGBSQLPGBA	ABYENLENSE MEYENLENSE SSSYSYGPHV SSTYSYGPHV SSTYSYGPHV KPGAPATETE	500 494 494 547 547
R15 Oko Okh R15 Oko	QQYRKLLXST QQYRKLLXST QGFFVAIIYC QGFFVAIIYC GGFFVAIIYC J	LVLMPLFGVH LVLMPLFGVH FCNGEVQAEI FCNGEVQAEI FCNGEVQAEI RAGLSLPLSP RGGLALSLSP	YIVFMATPYT YIVFMATPYT RKSWSRWTLA KKSWSRWTLA KKSWSRWTLA RLPPATT RLAPGAGASAWPCPSA	EVSGILWOVO EVSGILWOVO LDFKRKARSG LDFKRKARSG LDFKRKARSG NGBSOLPGBA NGBSOLPGBA NGBSOLPGBA LD	ABYENLINSF MEYENLINSF SSSYSYGPMV SSTYSYGPMV SSTYSYGPMV KPGAPATETE KHGSISENSL	500 494 494 547 547 544 515
R15 Oko Okh R15 Oko	QQYRKLLXST QQYRKLLXST QGFFVAIIYC QGFFVAIIYC J SHTSVTNVGP SHTSVTNVGP SHTSVTNVGP	LVLMPLFGVH LVLMPLFGVH FCNGEVQAEI FCNGEVQAEI FCNGEVQAEI RAGLSLPLSP	YIVFMATPYT YIVFMATPYT RKSWSRWTLA KKSWSRWTLA KKSWSRWTLA RLPPATT RLAPGAGASAWPCPSA	EVSGILWOVO EVSGILWOVO LDFKRKARSG LDFKRKARSG LDFKRKARSG NGBSQLPGBA NGBGQLPGYV LD RPPPLLOEGW	MEYEMLENSE MEYEMLENSE SSSYSYGPHV SSTYSYGPHV SSTYSYGPHV KPGAPATETE KHGSISENSL	500 494 494 547 547

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## FIG. 6

With I enzymes: SACI

February 27, 1992 18:30 ..

٠		GGGATCCSG	CGGCCCT	AGGCG	GTGGC	Gat	<u> </u>	SACC	:GCc	cđć	ato	cgca	ccc	ggc	ctg	gcg	61
	2	CCCTAGGGC	GCCGGGA	rccgc	CACCG	Ctá		Tş	CG	iàc	:220	ica:	359	ccg	gac		01
5						M	G	:	A	R	:	A	2	G	L	A	-
	<b>62</b>	ctcctgctc															121
		gaggacgag	acgacgg	ggcac	gagtc	gag	dc2:	cato	acad	cgad	ca	ccta	cgt	:cta	ctç	cag	
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	122	tactgattt	ctsetts	tctag	aagga	cça	cșt	<b>;</b> ;;	gCů:	agt:	ccg	ggt	cacq	gctt	ttt	gcc	
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	132	gagttect	caggac	tetee	à de ca	geo	àr3	tta	cct	tag	tct	gtt	226	tac	tgt		
5		t k e	v z ç	2 3	5 Y	s	=	::	Ξ	3	כ	ĸ	G	M	T	s	-
	242	gcgtccaca	tcaggga	ageco	aggaa	aga	itaa	dåc	atc	tạg	gaa	gct	cta	ccc1	ga	gtct +-	301
	276	:gczggtgt	agtocct	:==999	;coct	tet	att	ccç	taģ	acc	ctt	cga	gat	<b>333</b>	act	caga	
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	. 402	sedredge	cctcdgc	cacgg	acccç	tçt	tçt	cct	ţca(	ccc	ààs	tga	tgt	eget	cac	cacag	ľ

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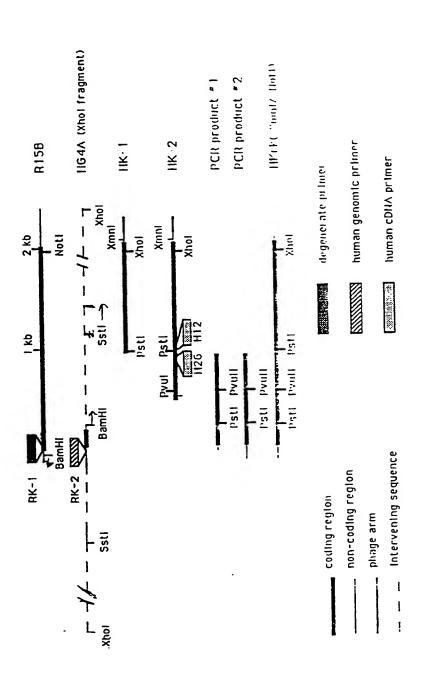


Fig. 7

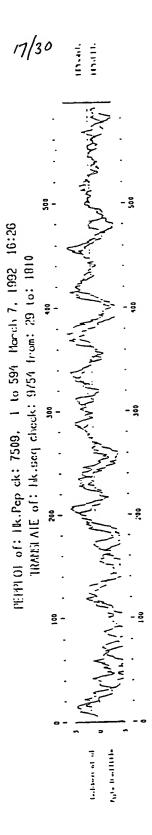


Fig.3

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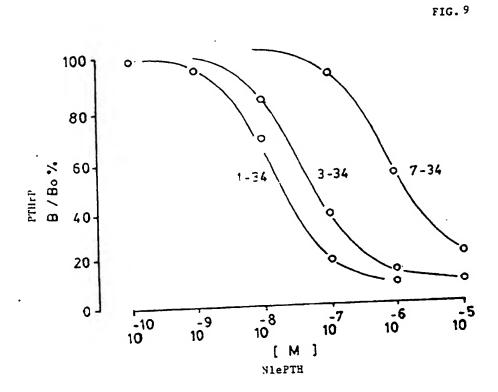
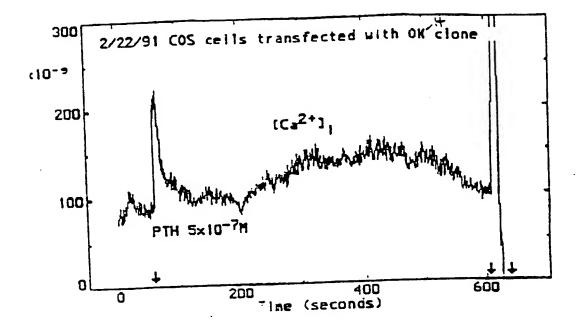
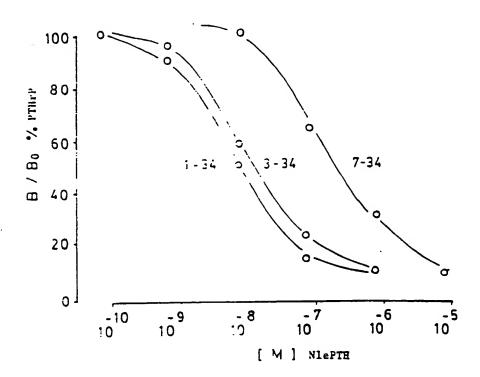


FIG. 10

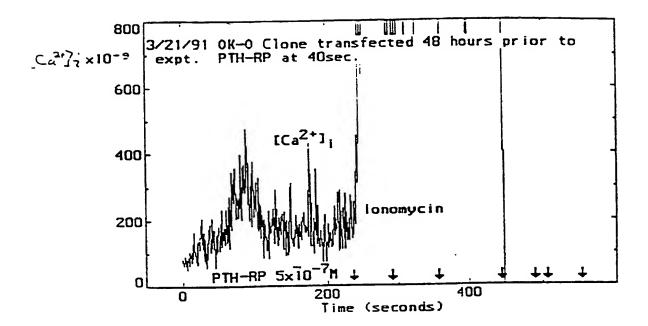


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Fig. 11



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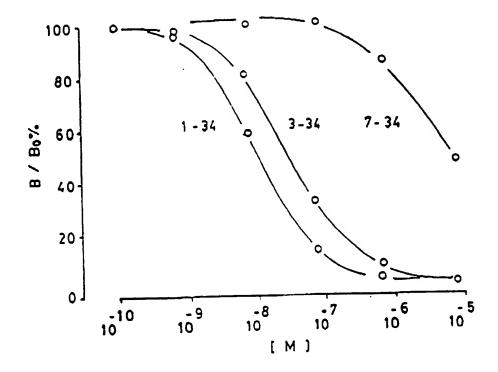
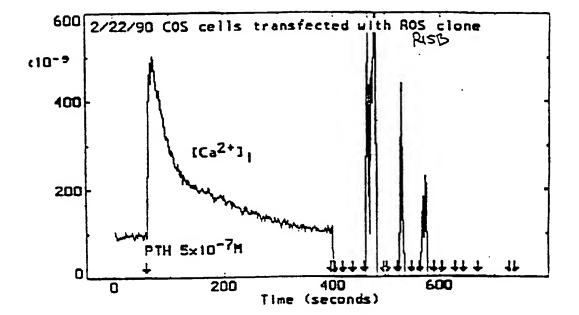


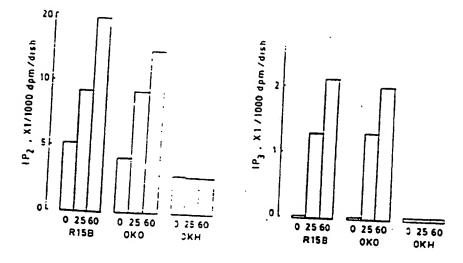
FIG. 13

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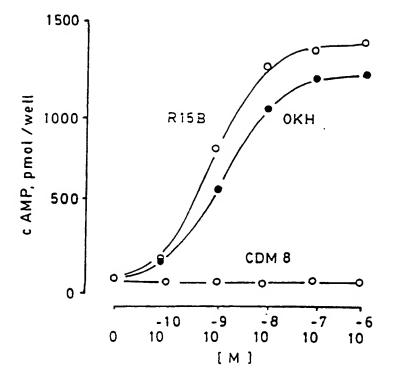
FIG. 14

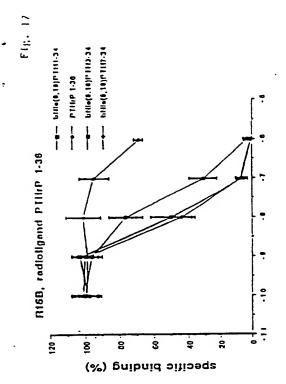


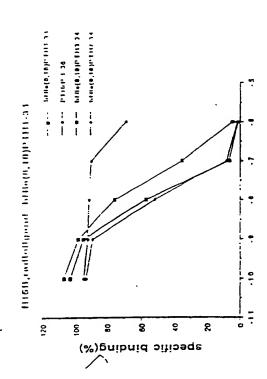
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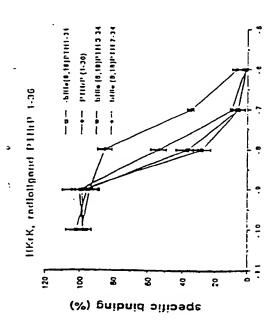


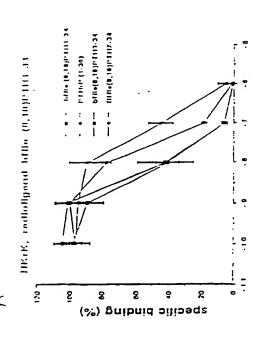
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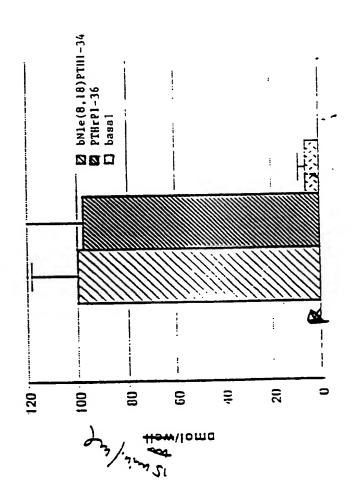






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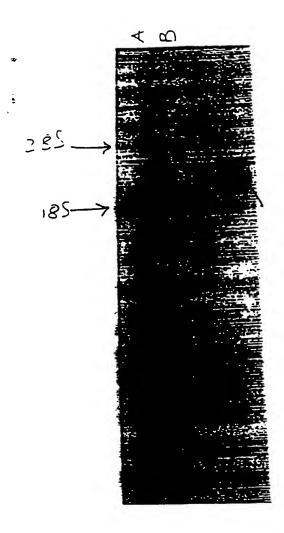


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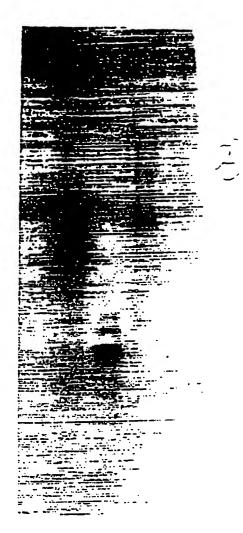




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Fiz. 20

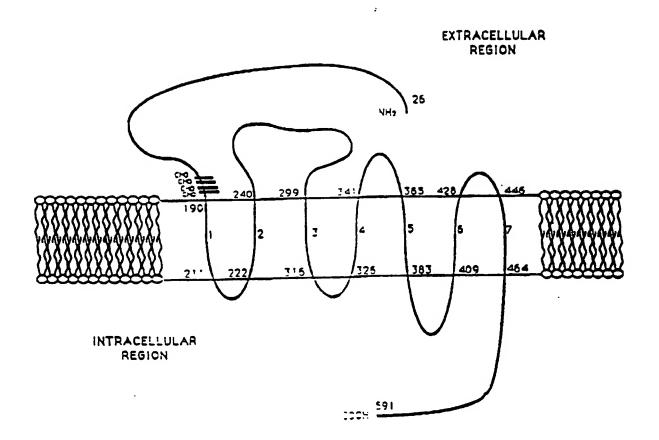


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Fig. 21

# RAT BONE PTH/PTHrP RECEPTOR



AMING ACID SEQUENCE OF 7 PUTATIVE TRANS-MEMBRANE REGIONS

VGYSMSLASLTVAVLILAY — HMHMFLSFMLRAAS-FVK — LVEGLYLHSLIFMAFFS

6

STLVLVPLFSVHYTVFMALP — VP:LASVVINFILFINIR — IFGWGLPAVFVAVWVGV

7

MLFNSFQGFFVAHY2FCN

### INTERNATIONAL SEARCH REPORT

International application No. PCT/US92/02821

A. CLASSIFICATION OF SUBJECT MATTER			
IPC(5) :Please See Extra Sheet. US CL :435/69.1, 240.2, 320.1; 536/27, 28, 29; 530/350, 387, 397, 399.			
According to International Patent Classification (IPC) or to both national classification and IPC			
B. FIELDS SEARCHED			
Minimum documentation searched (classification system followed by classification symbols)			
U.S. : APS AND COMMERCIAL DATABASES (DIALOG) 435/69.1, 240.2, 320.1; 536/27, 28, 29			
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched			
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) DIALOG AND ONLINE SEQUENCE SEARCH			
C. DOCUMENTS CONSIDERED TO BE RELEVANT			
Category*	Citation of document, with indication, where ap	propriate, of the relevant passages	Relevant to claim No.
X Y	TWENTY-SEVENTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY, VOLUME 105, NO. 4, PT. 2, ISSUED OCTOBER 1987, R. A. LUBEN ET AL., "MOLECULAR CLONING OF A PARATHYROID HORMONE RECEPTOR-RELATED MEMBRANE PROTEIN FROM MOUSE BONE CELLS", ENTIRE DOCUMENT.		
Y	THE JOURNAL OF BIOLOGICAL CHEMISTRY, VOL 265, NO. 1, ISSUED 05 JANUARY 1990, ABOU-SAMRA ET AL., "CHARACTERIZATION OF FULLY ACTIVE BIOTINYLATED PARATHYROID HORMONE ANALOGS", PAGES 58-62, ENTIRE DOCUMENT.		1-49
Y	BIOCHEMISTRY, VOLUME 29, NO. 30, ISSUED 31 JULY 1990, JUPPNER ET AL., "PREPARATION AND CHARACTERIZATION (N-(4-AZIDO-2-NITROPHENYL)ALA, TYR-36)-PATHYROID HORMONE RELATED PEPTIDE (1-36) AMIDE: A HIGH- AFFINITY, PARTIAL AGONIST HAVING HIGH CROSS-LINKING EFFICIENCY WITH ITS RECEPTOR ON ROS 17/2.8 CELLS", PAGES 6941-6946, ENTIRE DOCUMENT.		
Further documents are listed in the continuation of Box C. See patent family annex.			
• Special categories of cited documents:  'A' document defining the general state of the art which is not considered to be part of particular relevance  'A' document defining the general state of the art which is not considered to be part of particular relevance  'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention			
		"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
cited to establish the publication date of another citation or other special reason (as specified)  "O" document referring to an oral disclosure, use, exhibition or other means		"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
*P* do	the priority date claimed		
		Date of mailing of the international search report  31 JUL 1992/	
			<del>   </del>
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### INTERNATIONAL SEARCH REPORT

International application No. PCT/US92/02821

A. CLASSIFICATION OF SUBJECT MATTER: IPC (5):

C12P 21/06; C12N 5/00, 15/00; C07H 15/12, 17/00; C07K 3/00; A61K 35/14, 37/24, 37/36.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING